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(57) Abstract

Using the Ebola GP, NP, VP24, VP30, VP35 and VP40 virion proteins, a method and composition for use in inducing an immune response which is protective against infection with Ebola virus is described.

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TITLE OF THE INVENTION

Ebola Virion Proteins Expressed from Venezuelan Equine Encephalitis (VEE) Virus Replicons

INTRODUCTION

Ebola viruses, members of the family Filoviridae, are associated with outbreaks of highly lethal hemorrhagic fever in humans and nonhuman primates. The natural reservoir of the virus is unknown and there currently are no available vaccines or effective therapeutic treatments for filovirus infections. The genome of Ebola virus consists of a single strand of negative sense RNA that is approximately 19 kb in length. This RNA contains seven sequentially arranged genes that produce 8 mRNAs upon infection (Fig. 1). Ebola virions, like virions of other filoviruses, contain seven proteins: a surface glycoprotein (GP), a nucleoprotein (NP), four virion structural proteins (VP40, VP35, VP30, and VP24), and an RNA-dependent RNA polymerase (L) (Feldmann *et al.*(1992) *Virus Res.* **24**, 1-19; Sanchez *et al.*,(1993) *Virus Res.* **29**, 215-240; reviewed in Peters *et al.* (1996) *In* Fields Virology, Third ed. pp. 1161-1176. Fields, B. N., Knipe, D. M., Howley, P.M., *et al.* eds. Lippincott-Raven Publishers, Philadelphia). The glycoprotein of Ebola virus is unusual in that it is encoded in two open reading frames. Transcriptional editing is needed to express the transmembrane form that is incorporated into the virion (Sanchez *et al.* (1996) *Proc. Natl. Acad. Sci. USA* **93**, 3602-3607;

Volchkov *et al*, (1995) *Virology* **214**, 421-430. The unedited form produces a nonstructural secreted glycoprotein (sGP) that is synthesized in large amounts early during the course of infection. Little is known about the biological functions of these proteins and it is not known which antigens significantly contribute to protection and should therefore be used to induce an immune response.

Recent studies using rodent models to evaluate subunit vaccines for Ebola virus infection using recombinant vaccinia virus encoding Ebola virus GP (Gilligan *et al.*, (1997) *In* Vaccines 97, pp. 87-92. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.), or naked DNA constructs expressing either GP or sGP (Xu *et al.* (1998) *Nature Med.* **4**, 37-42) have demonstrated the protective efficacy of Ebola virus GP in guinea pigs. (All documents cited herein *supra* and *infra* are hereby incorporated in their entirety by reference thereto.) Additionally, Ebola virus NP and GP genes expressed from naked DNA vaccines (Vanderzanden *et al.*,(1998) *Virology* **246**, 134-144) have elicited protective immunity in BALB/c mice. However, successful vaccination of nonhuman primates with individual Ebola virus genes has not been demonstrated. Therefore, there exists a need for a vaccine which is efficacious for protection from Ebola virus infection.

SUMMARY OF THE INVENTION

The present invention satisfies the need discussed above. The present invention relates to a method and composition for use in inducing an immune response which is protective against infection with Ebola virus.

Because the biological functions of the individual Ebola virus proteins are not known and the immune mechanisms necessary for preventing and

clearing Ebola virus infection are not well understood, it was not clear which antigens significantly contribute to protection and should therefore be included in an eventual vaccine candidate to induce a protective immune response. We evaluated the ability of packaged Venezuelan equine encephalitis (VEE) virus replicons expressing GP, NP, VP40, VP35, VP30 and VP24 virion proteins of Ebola virus to elicit protective immunity in two strains of mice which differ at the major histocompatibility locus. There are no published reports of the VP proteins having been assayed as antigens for the production of an immune response in a mammal.

The VEE virus replicon (Vrep) is a genetically reorganized version of the VEE virus genome in which the structural protein genes are replaced with a gene from an immunogen of interest, such as the Ebola virus virion proteins. This replicon can be transcribed to produce a self-replicating RNA that can be packaged into infectious particles using defective helper RNAs that encode the glycoprotein and capsid proteins of the VEE virus. Since the packaged replicons do not encode the structural proteins, they are incapable of spreading to new cells and therefore undergo a single abortive round of replication in which large amounts of the inserted immunogen are made in the infected cells. The VEE virus replicon system is described in U.S. Patent to Johnston *et al.*, patent no. 5,792,462 issued on August 11, 1998.

For our purposes, each of the Ebola virus genes were individually inserted into a VEE virus replicon vector. The VP24, VP30, VP35, and VP40 genes of Ebola Zaire 1976 (Mayinga isolate) were cloned by reverse transcription of RNA from Ebola-infected Vero E6 cells and viral cDNAs were amplified using the polymerase chain reaction. The Ebola Zaire 1976 (Mayinga isolate) GP and NP genes were obtained from plasmids already containing these genes (Sanchez, A. *et al.*, (1989)

Virology 193, 81-91; Sanchez A. *et al.*, (1993) *Virus Res.* 29, 215-240) and were subcloned into the VEE replicon vector.

After characterization of the Ebola gene products expressed from the VEE replicon constructs in cell culture, these constructs were packaged into infectious VEE virus replicon particles (VRPs) and subcutaneously injected into BALB/c and C57BL/6 mice. As controls in these experiments, mice were also immunized with a VEE replicon expressing Lassa nucleoprotein (NP) as an irrelevant control antigen, or injected with PBS buffer alone. The results of this study demonstrate that VRPs expressing the Ebola GP, NP, VP24, VP30, VP35 or VP40 genes induced protection in mice and may provide protection in humans.

Therefore, it is one object of the present invention to provide a DNA fragment encoding each of the Ebola Zaire 1976 GP, NP, VP24, VP30, VP35, and VP40 virion proteins (SEQUENCE ID NOS. 1-7).

It is another object of the present invention to ~~provide the DNA fragments of the [illegible] virion~~ proteins in a recombinant vector. When the vector is an expression vector, the Ebola virion proteins GP, NP, VP24, VP30, VP35, and VP40 are produced.

It is yet another object of the present invention to provide a VEE virus replicon vector comprising a VEE virus replicon and a DNA fragment encoding any of the Ebola Zaire 1976 (Mayinga isolate) GP, NP, VP24, VP30, VP35, or VP40 proteins. The construct can be used as a nucleic acid vaccine or for the production of self replicating RNA.

[illegible] It is another object of the present invention to provide a self replicating RNA comprising the VEE virus replicon and any of the Ebola Zaire 1976

(Mayinga isolate) RNAs encoding the GP, NP, VP24, VP30, VP35, and VP40 proteins described above. The RNA can be used as a vaccine for protection from Ebola infection. When the RNA is packaged, a VEE virus replicon particle is produced.

It is another object of the present invention to provide infectious VEE virus replicon particles produced from the VEE virus replicon RNAs described above.

It is further an object of the invention to provide an immunological composition for the protection of subjects against Ebola virus infection, comprising VEE virus replicon particles containing the Ebola virus GP, NP, VP24, VP30, VP35, or VP40 proteins, or any combination of different VEE virus replicons each containing one or more different Ebola proteins selected from GP, NP, VP24, VP30, VP35 and VP40.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description and appended claims, and accompanying drawings where:

Figure 1 is a schematic description of the organization of the Ebola virus genome.

Figures 2A, 2B and 2C are schematic representations of the VEE replicon constructs containing Ebola genes.

Figure 3 shows the generation of VEE viral-like particles containing Ebola genes.

Figure 4 is an immunoprecipitation of Ebola proteins produced from replicon constructs.

DETAILED DESCRIPTION

In the description that follows, a number of terms used in recombinant DNA, virology and immunology are extensively utilized. In order to provide a clearer and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

Filoviruses. The filoviruses (e.g. Ebola Zaire 1976) cause acute hemorrhagic fever characterized by high mortality. Humans can contract filoviruses by infection in endemic regions, by contact with imported primates, and by performing scientific research with the virus. However, there currently are no available vaccines or effective therapeutic treatments for filovirus infection. The virions of filoviruses contain seven proteins: a membrane-anchored glycoprotein (GP), a nucleoprotein (NP), an RNA-dependent RNA polymerase (L), and four virion structural proteins (VP24, VP30, VP35, and VP40). Little is known about the biological functions of these proteins and it is not known which antigens significantly contribute to protection and should therefore be used in an eventual vaccine candidate.

Replicon. A replicon is equivalent to a full-length virus from which all of the viral structural proteins have been deleted. A multiple cloning site can be inserted downstream of the 26S promoter into the site previously occupied by the structural protein genes. Virtually any heterologous gene may be inserted into this cloning site. The RNA that is transcribed from the replicon is capable of replicating and expressing viral proteins in a manner that is similar to that seen with the full-length infectious virus clone. However, in lieu of the viral structural proteins, the heterologous antigen is expressed from

the 26S promoter in the replicon. This system does not yield any progeny virus particles because there are no viral structural proteins available to package the RNA into particles.

Particles which appear structurally identical to virus particles can be produced by supplying structural protein RNAs *in trans* for packaging of the replicon RNA. This is typically done with two defective helper RNAs which encode the structural proteins. One helper consists of a full length infectious clone from which the nonstructural protein genes and the glycoprotein genes are deleted. This helper retains only the terminal nucleotide sequences, the promoter for subgenomic mRNA transcription and the sequences for the viral nucleocapsid protein. The second helper is identical to the first except that the nucleocapsid gene is deleted and only the glycoprotein genes are retained. The helper RNAs are transcribed *in vitro* and are co-transfected with replicon RNA. Because the replicon RNA retains the sequences for packaging by the nucleocapsid protein, and because the helpers lack these sequences, only the replicon RNA is packaged by the viral structural proteins. The packaged replicon particles are released from the host cell and can then be purified and inoculated into animals. The packaged replicon particles will have a tropism similar to the parent virus. The packaged replicon particles will infect cells and initiate a single round of replication, resulting in the expression of only the virus nonstructural proteins and the product of the heterologous gene that was cloned in the place of the virus structural proteins. In the absence of RNA encoding the virus structural proteins, no progeny virus particles can be produced from the cells infected by packaged replicon particles.

The Venezuelan equine encephalitis (VEE) virus replicon is a genetically reorganized version of the

VEE virus genome in which the genes encoding the VEE structural proteins are replaced with a heterologous gene of interest. In the present invention, the heterologous genes are the GP, NP, or VP virion proteins from the Ebola virus. The result is a self-replicating RNA that can be packaged into infectious particles using defective helper RNAs that encode the glycoprotein and capsid proteins of the VEE virus. The replicon and its use is further described in U.S. Patent no 5,792,462 issued to Johnston *et al.* on August 11, 1998.

Subject. Includes both human, animal, e.g., horse, donkey, pig, mouse, hamster, monkey, chicken, and insect such as mosquito.

In one embodiment, the present invention relates to DNA fragments which encode any of the Ebola Zaire 1976 (Mayinga isolate) GP, NP, VP24, VP30, VP35, and VP40 proteins. The GP and NP genes of Ebola Zaire were previously sequenced by Sanchez *et al.* (1993, *supra*) and have been deposited in GenBank (accession number L11365). A plasmid encoding the VEE replicon vector containing a unique ClaI site downstream from the 26S promoter was described previously (Davis, N. L. *et al.*, (1996) *J. Virol.* **70**, 3781-3787; Pushko, P. *et al.* (1997) *Virology* **239**, 389-401). The Ebola GP and NP genes from the Ebola Zaire 1976 virus were derived from PS64- and PGEM3ZF(-)-based plasmids (Sanchez, A. *et al.* (1989) *Virology* **170**, 81-91; Sanchez, A. *et al.* (1993) *Virus Res.* **29**, 215-240). From these plasmids, the BamHI-EcoRI (2.3 kb) and BamHI-KpnI (2.4 kb) fragments containing the NP and GP genes, respectively, were subcloned into a shuttle vector that had been digested with BamHI and EcoRI (Davis *et al.* (1996) *supra*; Grieder, F. B. *et al.* (1995) *Virology* **206**, 994-1006). For cloning of the GP gene, overhanging ends produced by KpnI (in the GP fragment) and EcoRI (in the shuttle vector) were made blunt by incubation with T4 DNA polymerase according to methods

known in the art. From the shuttle vector, GP or NP genes were subcloned as ClaI-fragments into the ClaI site of the replicon clone, resulting in plasmids encoding the GP or NP genes in place of the VEE structural protein genes downstream from the VEE 26S promoter.

The VP genes of Ebola Zaire were previously sequenced by Sanchez *et al.* (1993, *supra*) and have been deposited in GenBank (accession number L11365). The VP genes of Ebola used in the present invention were cloned by reverse transcription of RNA from Ebola-infected Vero E6 cells and subsequent amplification of viral cDNAs using the polymerase chain reaction. First strand synthesis was primed with oligo dT (Life Technologies). Second strand synthesis and subsequent amplification of viral cDNAs were performed with gene-specific primers (SEQ ID NOS:8-16). The primer sequences were derived from the GenBank deposited sequences and were designed to contain a ClaI restriction site for cloning the amplified VP genes into the ClaI site of the replicon vector. The letters and numbers in bold print indicate Ebola gene sequences in the primers and the corresponding location numbers based on the GenBank depositied sequences.

VP24: (1) forward primer is
5'-GGGATCGAT**CTCCAGACACCAAGCAAGACC**-3'(SEQ ID NO:8)
(10,311-10,331)
(2) reverse primer is
5'-GGGATCGAT**GAGTCAGCATATATGAGTTAGCTC**-3' (SEQ ID NO:9)
(11,122-11,145)

VP30: (1) forward primer is
5'-CCCATCG**ATCAGATCTGCGAACCGGTAGAG**-3' SEQ ID NO:10)
(8408-8430)
(2) reverse primer is
5'-CCCATCGAT**GTACCCTCATCAGACCATGAGC**-3' (SEQ ID NO:11)

(9347-9368)

VP35: (1) forward primer is 5'-GGGATCGAT**AGAAAAGCTGGTCTAACAAGATGA**-3'(SEQ ID NO:12)

(3110-3133)

(2) reverse primer is 5'-CCCATCGAT**CTCACAAGTGTATCATTAATGTAACGT**-3'(SEQ ID NO:13) **(4218-4244)**

VP40: (1) forward primer is 5'-CCCATCGAT**CCTACCTCGGCTGAGAGAGTG**-3'(SEQ ID NO:14)

(4408-4428)

(2) reverse primer is 5'-CCCATCGAT**ATGTTATGCACTATCCCTGAGAAG**-3'(SEQ ID NO:15)

(5495-5518)

VP30 #2:

(1) forward primer as for VP30 above

(2) reverse primer is 5'-CCCATCGATCTGTTAGGGTTGTATCATACC-3'(SEQ ID NO:16)

The Ebola virus genes cloned into the VEE replicon were sequenced. Changes in the DNA sequence relative to the sequence published by Sanchez *et al.* (1993) are described relative to the nucleotide (nt) sequence number from GenBank (accession number L11365).

The nucleotide sequence we obtained for Ebola virus GP (SEQ ID NO:1) differed from the GenBank sequence by a transition from A to G at nt 8023. This resulted in a change in the amino acid sequence from Ile to Val at position 662 (SEQ ID NO: 17).

The nucleotide sequence we obtained for Ebola virus NP (SEQ ID NO:2) differed from the GenBank sequence at the following 4 positions: insertion of a C residue between nt 973 and 974, deletion of a G residue at nt 979, transition from C to T at nt 1307, and a transversion from A to C at nt 2745. These changes resulted in a change in the protein sequence

from Arg to Glu at position 170 and a change from Leu to Phe at position 280 (SEQ ID NO: 18).

The Ebola virus VP24 nucleotide sequence (SEQ ID NO:3) differed from the GenBank sequence at 6 positions, resulting in 3 nonconservative changes in the amino acid sequence. The changes in the DNA sequence of VP24 consisted of a transversion from G to C at nt 10795, a transversion from C to G at nt 10796, a transversion from T to A at nt 10846, a transversion from A to T at nt 10847, a transversion from C to G at nt 11040, and a transversion from C to G at nt 11041. The changes in the amino acid sequence of VP24 consisted of a Cys to Ser change at position 151, a Leu to His change at position 168, and a Pro to Gly change at position 233 (SEQ ID NO: 19).

Two different sequences for the Ebola virus VP30 gene, VP30 and VP30#2 (SEQ ID NOS: 4 and 7) are included. Both of these sequences differ from the GenBank sequence by the insertion of an A residue in the upstream noncoding sequence between nt 8469 and 8470 and an insertion of a T residue between nt 9275 and 9276 that results in a change in the open reading frame of VP30 and VP30#2 after position 255 (SEQ ID NOS: 20 and 23). As a result, the C-terminus of the VP30 protein differs significantly from that previously reported. In addition to these 2 changes, the VP30#2 nucleic acid in SEQ ID NO:7 contains a conservative transition from T to C at nt 9217. Because the primers originally used to clone the VP30 gene into the replicon were designed based on the GenBank sequence, the first clone that we constructed (SEQ ID NO: 4) did not contain what we believe to be the authentic C-terminus of the protein. Therefore, in the absence of the VP30 stop codon, the C-terminal codon was replaced with 37 amino acids derived from the vector sequence. The resulting VP30 construct therefore differed from the GenBank sequence in that it contained 32 amino acids of VP30 sequence

(positions 256 to 287, SEQ ID NO:20) and 37 amino acids of irrelevant sequence (positions 288 to 324, SEQ ID NO:20) in the place of the C-terminal 5 amino acids reported in GenBank. However, inclusion of 37 amino acids of vector sequence in place of the C-terminal amino acid (Pro, SEQ ID NO: 23) did not inhibit the ability of the protein to serve as a protective antigen in BALB/c mice. We are currently examining the ability of the new VEE replicon construct, which we believe contains the authentic C-terminus of VP30 (VP30#2, SEQ ID NO: 23), to protect mice against a lethal Ebola challenge.

The nucleotide sequence for Ebola virus VP35 (SEQ ID NO:5) differed from the GenBank sequence by a transition from T to C at nt 4006, a transition from T to C at nt 4025, and an insertion of a T residue between nt 4102 and 4103. These sequence changes resulted in a change from a Ser to a Pro at position 293 and a change from Phe to Ser at position 299 (SEQ ID NO: 21). The insertion of the T residue resulted in a change in the open reading frame of VP35 from that previously reported by Sanchez *et al.* (1993) following amino acid number 324. As a result, Ebola virus VP35 encodes a protein of 340 amino acids, where amino acids 325 to 340 (SEQ ID NO: 21) differ from and replace the C-terminal 27 amino acids of the previously published sequence.

Sequencing of VP30 and VP35 was also performed on RT/PCR products from RNA derived from cells that were infected with Ebola virus 1976, Ebola virus 1995 or the mouse-adapted Ebola virus. The changes noted above for the Vrep constructs were also found in these Ebola viruses. Thus, we believe that these changes are real events and not artifacts of cloning.

The Ebola virus VP40 nucleotide sequence (SEQ ID NO:6) differed from the GenBank sequence by a transversion from a C to G at nt 4451 and a transition from a G to A at nt 5081. These sequence changes did

not alter the protein sequence of VP40 (SEQ ID NO: 22) from that of the published sequence.

DNA or polynucleotide sequences to which the invention also relates include sequences of at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10-12 nucleotides, most preferably at least about 15-20 nucleotides corresponding, i.e., homologous to or complementary to, a region of the Ebola nucleotide sequences described above. Preferably, the sequence of the region from which the polynucleotide is derived is homologous to or complementary to a sequence which is unique to the Ebola genes. Whether or not a sequence is unique to the Ebola gene can be determined by techniques known to those of skill in the art. For example, the sequence can be compared to sequences in databanks, e.g., GenBank and compared by DNA:DNA hybridization. Regions from which typical DNA sequences may be derived include but are not limited to, for example, regions encoding specific epitopes, as well as non-transcribed and/or non-translated regions.

The derived polynucleotide is not necessarily physically derived from the nucleotide sequences shown in SEQ ID NO:1-7, but may be generated in any manner, including for example, chemical synthesis or DNA replication or reverse transcription or transcription, which are based on the information provided by the sequence of bases in the region(s) from which the polynucleotide is derived. In addition, combinations of regions corresponding to that of the designated sequence may be modified in ways known in the art to be consistent with an intended use. The sequences of the present invention can be used in diagnostic assays such as hybridization assays and polymerase chain reaction assays, for example, for the discovery of other Ebola sequences.

In another embodiment, the present invention relates to a recombinant DNA molecule that includes a

vector and a DNA sequence as described above. The vector can take the form of a plasmid, a eukaryotic expression vector such as pcDNA3.1, pRcCMV2, pZeoSV2,or pCDM8, which are available from Invitrogen, or a virus vector such as baculovirus vectors, retrovirus vectors or adenovirus vectors, alphavirus vectors, and others known in the art.

In a further embodiment, the present invention relates to host cells stably transformed or transfected with the above-described recombinant DNA constructs. The host cell can be prokaryotic (for example, bacterial), lower eukaryotic (for example, yeast or insect) or higher eukaryotic (for example, all mammals, including but not limited to mouse and human). Both prokaryotic and eukaryotic host cells may be used for expression of the desired coding sequences when appropriate control sequences which are compatible with the designated host are used.

Among prokaryotic hosts, *E. coli* is the most frequently used host cell for expression. General control sequences for prokaryotes include promoters and ribosome binding sites. Transfer vectors compatible with prokaryotic hosts are commonly derived from a plasmid containing genes conferring ampicillin and tetracycline resistance (for example, pBR322) or from the various pUC vectors, which also contain sequences conferring antibiotic resistance. These antibiotic resistance genes may be used to obtain successful transformants by selection on medium containing the appropriate antibiotics. Please see e.g., Maniatis, Fitsch and Sambrook, Molecular Cloning: A Laboratory Manual (1982) or DNA Cloning, Volumes I and II (D. N. Glover ed. 1985) for general cloning methods. The DNA sequence can be present in the vector operably linked to sequences encoding an IgG molecule, an adjuvant, a carrier, or an agent for

aid in purification of Ebola proteins, such as glutathione S-transferase.

In addition, the Ebola virus gene products can also be expressed in eukaryotic host cells such as yeast cells and mammalian cells. *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis*, and *Pichia pastoris* are the most commonly used yeast hosts. Control sequences for yeast vectors are known in the art. Mammalian cell lines available as hosts for expression of cloned genes are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), such as CHO cells, Vero cells, baby hamster kidney (BHK) cells and COS cells, to name a few. Suitable promoters are also known in the art and include viral promoters such as that from SV40, Rous sarcoma virus (RSV), adenovirus (ADV), bovine papilloma virus (BPV), and cytomegalovirus (CMV). Mammalian cells may also require terminator sequences, poly A addition sequences, enhancer sequences which increase expression, or sequences which cause amplification of the gene. These sequences are known in the art.

The transformed or transfected host cells can be used as a source of DNA sequences described above. When the recombinant molecule takes the form of an expression system, the transformed or transfected cells can be used as a source of the protein described below.

In another embodiment, the present invention relates to Ebola virion proteins such as GP having an amino acid sequence corresponding to SEQ ID NO:17 encompassing 676 amino acids, NP, having an amino acid sequence corresponding to SEQ ID NO:18 encompassing 739 amino acids, VP24, having an amino acid sequence corresponding to SEQ ID NO:19 encompassing 251 amino acids, VP30, having an amino acid sequence corresponding SEQ ID NO:20 encompassing 324 amino acids, VP35, having an amino acid sequence

corresponding to SEQ ID NO:21 encompassing 340 amino acids, and VP40, having an amino acid sequence corresponding to SEQ ID NO:22, encompassing 326 amino acids, and VP30#2, having an amino acid sequence corresponding to SEQ ID NO:23 encompassing 288 amino acids, or any allelic variation of the amino acid sequences. By allelic variation is meant a natural or synthetic change in one or more amino acids which occurs between different serotypes or strains of Ebola virus and does not affect the antigenic properties of the protein. There are different strains of Ebola (Zaire 1976, Zaire 1995, Reston, Sudan, and Ivory Coast). The NP and VP genes of these different viruses have not been sequenced. It would be expected that these proteins would have homology among different strains and that vaccination against one Ebola virus strain might afford cross protection to other Ebola virus strains.

A polypeptide or amino acid sequence derived from any of the amino acid sequences in SEQ ID NO:17, 18, 19, 20, 21, 22, and 23 refers to a polypeptide having an amino acid sequence identical to that of a polypeptide encoded in the sequence, or a portion thereof wherein the portion consists of at least 2-5 amino acids, preferably at least 8-10 amino acids, and more preferably at least 11-15 amino acids, or which is immunologically identifiable with a polypeptide encoded in the sequence.

A recombinant or derived polypeptide is not necessarily translated from a designated nucleic acid sequence, or the DNA sequence found in GenBank accession number L11365. It may be generated in any manner, including for example, chemical synthesis, or expression from a recombinant expression system.

When the DNA or RNA sequences described above are in a replicon expression system, such as the VEE replicon described above, the proteins can be expressed *in vivo*. The DNA sequence for any of the

GP, NP, VP24, VP30, VP35, and VP40 virion proteins can be cloned into the multiple cloning site of a replicon such that transcription of the RNA from the replicon yields an infectious RNA encoding the Ebola protein or proteins of interest (see Figure 2A, 2B and 2C). The replicon constructs include Ebola virus GP (SEQ ID NO:1) cloned into a VEE replicon (VRepEboGP), Ebola virus NP (SEQ ID NO:2) cloned into a VEE replicon (VRepEboNP), Ebola virus VP24 (SEQ ID NO:3) cloned into a VEE replicon (VRepEboVP24), Ebola virus VP30 (SEQ ID NO:4) or VP30#2 (SEQ ID NO:7) cloned into a VEE replicon (VRepEboVP30 or VRepEboVP30(#2)), Ebola virus VP35 (SEQ ID NO:5) cloned into a VEE replicon (VRepEboVP35), and Ebola virus VP40 (SEQ ID NO:6) cloned into a VEE replicon (VRepEboVP40). The replicon DNA or RNA can be used as a vaccine for inducing protection against infection with Ebola. Use of helper RNAs containing sequences necessary for packaging of the viral replicon transcripts will result in the production of virus-like particles containing replicon RNAs (Figure 3). These packaged replicons will infect host cells and initiate a single round of replication resulting in the expression of the Ebola proteins in said infected cells. The packaged replicon constructs (i.e. VEE virus replicon particles, VRP) include those that express Ebola virus GP (EboGPVRP), Ebola virus NP (EboNPVRP), Ebola virus VP24 (EboVP24VRP), Ebola virus VP30 (EboVP30VRP or EboVP30VRP(#2)), Ebola virus VP35 (EboVP35VRP), and Ebola virus VP40 (EboVP40VRP).

In another embodiment, the present invention relates to RNA molecules resulting from the transcription of the constructs described above. The RNA molecules can be prepared by *in vitro* transcription using methods known in the art and described in the Examples below. Alternatively, the RNA molecules can be produced by transcription of the constructs *in vivo*, and isolating the RNA. These and other methods for

obtaining RNA transcripts of the constructs are known in the art. Please see Current Protocols in Molecular Biology. Frederick M. Ausubel et al. (eds.), John Wiley and Sons, Inc. The RNA molecules can be used, for example, as a direct RNA vaccine, or to transfect cells along with RNA from helper plasmids, one of which expresses VEE glycoproteins and the other VEE capsid proteins, as described above, in order to obtain replicon particles.

In a further embodiment, the present invention relates to a method of producing the recombinant or fusion protein which includes culturing the above-described host cells under conditions such that the DNA fragment is expressed and the recombinant or fusion protein is produced thereby. The recombinant or fusion protein can then be isolated using methodology well known in the art. The recombinant or fusion protein can be used as a vaccine for immunity against infection with Ebola or as a diagnostic tool for detection of Ebola infection.

In another embodiment, the present invention relates to antibodies specific for the above-described recombinant proteins (or polypeptides). For instance, an antibody can be raised against a peptide having the amino acid sequence of any of SEQ ID NO:17-25, or against a portion thereof of at least 10 amino acids, preferably, 11-15 amino acids. Persons with ordinary skill in the art using standard methodology can raise monoclonal and polyclonal antibodies to the protein(or polypeptide) of the present invention, or a unique portion thereof. Materials and methods for producing antibodies are well known in the art (see for example Goding, *In* Monoclonal Antibodies: Principles and Practice, Chapter 4, 1986).

In a further embodiment, the present invention relates to a method of detecting the presence of antibodies against Ebola virus in a sample. Using

standard methodology well known in the art, a diagnostic assay can be constructed by coating on a surface (i.e. a solid support for example, a microtitration plate, a membrane (e.g. nitrocellulose membrane) or a dipstick), all or a unique portion of any of the Ebola proteins described above or any combination thereof, and contacting it with the serum of a person or animal suspected of having Ebola. The presence of a resulting complex formed between the Ebola protein(s) and serum antibodies specific therefor can be detected by any of the known methods common in the art, such as fluorescent antibody spectroscopy or colorimetry. This method of detection can be used, for example, for the diagnosis of Ebola infection and for determining the degree to which an individual has developed virus-specific Abs after administration of a vaccine.

In yet another embodiment, the present invention relates to a method for detecting the presence of Ebola virion proteins in a sample. Antibodies against GP, NP, and the VP proteins could be used for diagnostic assays. Using standard methodology well known in the art, a diagnostics assay can be constructed by coating on a surface (i.e. a solid support, for example, a microtitration plate or a membrane (e.g. nitrocellulose membrane)), antibodies specific for any of the Ebola proteins described above, and contacting it with serum or a tissue sample of a person suspected of having Ebola infection. The presence of a resulting complex formed between the protein or proteins in the serum and antibodies specific therefor can be detected by any of the known methods common in the art, such as fluorescent antibody spectroscopy or colorimetry. This method of detection can be used, for example, for the diagnosis of Ebola virus infection.

In another embodiment, the present invention relates to a diagnostic kit which contains any

combination of the Ebola proteins described above and ancillary reagents that are well known in the art and that are suitable for use in detecting the presence of antibodies to Ebola in serum or a tissue sample. Tissue samples contemplated can be from monkeys, humans, or other mammals.

In yet another embodiment, the present invention relates to DNA or nucleotide sequences for use in detecting the presence of Ebola virus using the reverse transcription-polymerase chain reaction (RT-PCR). The DNA sequence of the present invention can be used to design primers which specifically bind to the viral RNA for the purpose of detecting the presence of Ebola virus or for measuring the amount of Ebola virus in a sample. The primers can be any length ranging from 7 to 400 nucleotides, preferably at least 10 to 15 nucleotides, or more preferably 18 to 40 nucleotides. Reagents and controls necessary for PCR reactions are well known in the art. The amplified products can then be analyzed for the presence of viral sequences, for example by gel fractionation, with or without hybridization, by radiochemistry, and immunochemistry techniques.

In yet another embodiment, the present invention relates to a diagnostic kit which contains PCR primers specific for Ebola virus and ancillary reagents for use in detecting the presence or absence of Ebola in a sample using PCR. Samples contemplated can be obtained from human, animal, e.g., horse, donkey, pig, mouse, hamster, monkey, or other mammals, birds, and insects, such as mosquitoes.

In another embodiment, the present invention relates to an Ebola vaccine comprising VRPs that express one or more of the Ebola proteins described above. The vaccine is administered to a subject wherein the replicon is able to initiate one round of replication producing the Ebola proteins to which a

protective immune response is initiated in said subject.

It is likely that the protection afforded by these genes is due to both the humoral (antibodies (Abs)) and cellular (cytotoxic T cells (CTLs)) arms of the immune system. Protective immunity induced to a specific protein may comprise humoral immunity, cellular immunity, or both. The only Ebola virus protein known to be on the outside of the virion is the GP. The presence of GP on the virion surface makes it a likely target for GP-specific Abs that may bind either extracellular virions or infected cells expressing GP on their surfaces. Serum transfer studies in this invention demonstrate that Abs that recognize GP protect mice against lethal Ebola virus challenge.

In contrast, transfer of Abs specific for NP, VP24, VP30, VP35, or VP40 did not protect mice against lethal Ebola challenge. This data, together with the fact that these are internal virion proteins that are not readily accessible to Abs on either extracellular virions or the surface of infected cells, suggest that the protection induced in mice by these proteins is mediated by CTLs.

CTLs can bind to and lyse virally infected cells. This process begins when the proteins produced by cells are routinely digested into peptides. Some of these peptides are bound by the class I or class II molecules of the major histocompatability complex (MHC), which are then transported to the cell surface. During virus infections, viral proteins produced within infected cells also undergo this process. CTLs that have receptors that bind to both a specific peptide and the MHC molecule holding the peptide lyse the peptide-bearing cell, thereby limiting virus replication. Thus, CTLs are characterized as being specific for a particular peptide and restricted to a class I or class II MHC molecule.

CTLs may be induced against any of the Ebola virus proteins, as all of the viral proteins are produced and digested within the infected cell. Thus, protection to Ebola virus could involve CTLs against GP, NP, VP24, VP30, VP35, and/or VP40. It is especially noteworthy that the VP proteins varied in their protective efficacy when tested in genetically inbred mice that differ at the MHC locus. This, together with the inability to demonstrate a role for Abs in protection induced by the VP proteins, strongly supports a role for CTLs. These data also suggest that an eventual vaccine candidate may include several Ebola virus proteins, or several CTL epitopes, capable of inducing broad protection in outbred populations (e.g. people). We have identified two sequences recognized by CTLs. They are Ebola virus NP SEQ ID NO:24 and Ebola virus VP24 SEQ ID NO:25. Testing is in progress to identify the role of CTLs in protection induced by each of these Ebola virus proteins and to define the minimal sequence requirements for the protective response. The CTL assay is well known in the art.

An eventual vaccine candidate might comprise these CTL sequences and others. These might be delivered as synthetic peptides, or as fusion proteins, alone or co-administered with cytokines and/or adjuvants or carriers safe for human use, e.g. aluminum hydroxide, to increase immunogenicity. In addition, sequences such as ubiquitin can be added to increase antigen processing for more effective CTL responses.

In yet another embodiment, the present invention relates to a method for providing immunity against Ebola virus, said method comprising administering one or more VRPs expressing any combination of the GP, NP, VP24, VP30 or VP30#2, VP35 and VP40 Ebola proteins to a subject such that a protective immune reaction is generated.

Vaccine formulations of the present invention comprise an immunogenic amount of a VRP, such as for example EboVP24VRP described above, or, for a multivalent vaccine, a combination of replicons, in a pharmaceutically acceptable carrier. An "immunogenic amount" is an amount of the VRP(s) sufficient to evoke an immune response in the subject to which the vaccine is administered. An amount of from about 10^4-10^8 focus-forming units per dose is suitable, depending upon the age and species of the subject being treated. The subject may be inoculated 2-3 times. Exemplary pharmaceutically acceptable carriers include, but are not limited to, sterile pyrogen-free water and sterile pyrogen-free physiological saline solution.

Administration of the VRPs disclosed herein may be carried out by any suitable means, including parenteral injection (such as intraperitoneal, subcutaneous, or intramuscular injection), *in ovo* injection of birds, orally, or by topical application of the virus (typically carried in a pharmaceutical formulation) to an airway surface. Topical application of the virus to an airway surface can be carried out by intranasal administration (e.g., by use of dropper, swab, or inhaler which deposits a pharmaceutical formulation intranasally). Topical application of the virus to an airway surface can also be carried out by inhalation administration, such as by creating respirable particles of a pharmaceutical formulation (including both solid particles and liquid particles) containing the replicon as an aerosol suspension, and then causing the subject to inhale the respirable particles. Methods and apparatus for administering respirable particles of pharmaceutical formulations are well known, and any conventional technique can be employed. Oral administration may be in the form of an ingestable liquid or solid formulation.

When the replicon RNA or DNA is used as a vaccine, the replicon RNA or DNA can be administered directly using techniques such as delivery on gold beads (gene gun), delivery by liposomes, or direct injection, among other methods known to people in the art. Any one or more DNA constructs or replicating RNA described above can be use in any combination effective to elicit an immunogenic response in a subject. Generally, the nucleic acid vaccine administered may be in an amount of about 1-5 ug of nucleic acid per dose and will depend on the subject to be treated, capacity of the subject's immune system to develop the desired immune response, and the degree of protection desired. Precise amounts of the vaccine to be administered may depend on the judgement of the practitioner and may be peculiar to each subject and antigen.

The vaccine may be given in a single dose schedule, or preferably a multiple dose schedule in which a primary course of vaccination may be with 1-10 separate doses, followed by other doses given at subsequent time intervals required to maintain and or reinforce the immune response, for example, at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. Examples of suitable immunization schedules include: (i) 0, 1 months and 6 months, (ii) 0, 7 days and 1 month, (iii) 0 and 1 month, (iv) 0 and 6 months, or other schedules sufficient to elicit the desired immune responses expected to confer protective immunity, or reduce disease symptoms, or reduce severity of disease.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors and thought to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of

skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

The following MATERIALS AND METHODS were used in the examples that follow.

Cells lines and viruses

BHK (ATCC CCL 10), Vero 76 (ATCC CRL 1587), and Vero E6 (ATCC CRL 1586) cell lines were maintained in minimal essential medium with Earle's salts, 5-10% fetal bovine serum, and 50 μg/mL gentamicin sulfate. For CTL assays, EL4 (ATCC TIB39), L5178Y (ATCC CRL 1723) and P815 (ATCC TIB64) were maintained in Dulbecco's minimal essential medium supplemented with 5-10% fetal bovine serum and antibiotics.

A stock of the Zaire strain of Ebola virus originally isolated from a patient in the 1976 outbreak (Mayinga) and passaged intracerebrally 3 times in suckling mice and 2 times in Vero cells was adapted to adult mice through serial passage in progressively older suckling mice (Bray *et al.*, (1998) *J. Infect. Dis.* **178**, 651-661). A plaque-purified ninth-mouse-passage isolate which was uniformly lethal for adult mice ("mouse-adapted virus") was propagated in Vero E6 cells, aliquotted, and used in all mouse challenge experiments and neutralization assays.

A stock of the Zaire strain of Ebola 1976 virus was passaged spleen to spleen in strain 13 guinea pigs four times. This guinea pig-adapted strain was used to challenge guinea pigs.

Construction and packaging of recombinant VEE virus replicons (VRPs)

Replicon RNAs were packaged into VRPs as described (Pushko *et al.*, 1997, supra). Briefly, capped replicon RNAs were produced *in vitro* by T7 run-

off transcription of NotI-digested plasmid templates using the RiboMAX T7 RNA polymerase kit (Promega). BHK cells were co-transfected with the replicon RNAs and the 2 helper RNAs expressing the structural proteins of the VEE virus. The cell culture supernatants were harvested approximately 30 hours after transfection and the replicon particles were concentrated and purified by centrifugation through a 20% sucrose cushion. The pellets containing the packaged replicon particles were suspended in PBS and the titers were determined by infecting Vero cells with serial dilutions of the replicon particles and enumerating the infected cells by indirect immunofluorescence with antibodies specific for the Ebola proteins.

Immunoprecipitation of Ebola virus proteins expressed from VEE virus replicons

BHK cells were transfected with either the Ebola virus GP, NP, VP24, VP30, VP35, or VP40 replicon RNAs. At 24 h post-transfection, the culture medium was replaced with minimal medium lacking cysteine and methionine, and proteins were labeled for 1 h with ^{35}S-labeled methionine and cysteine. Cell lysates or supernatants (supe) were collected and immunoprecipitated with polyclonal rabbit anti-Ebola virus serum bound to protein A beads. ^{35}S-labeled Ebola virus structural proteins from virions grown in Vero E6 cells were also immunoprecipitated as a control for each of the virion proteins. Immunoprecipitated proteins were resolved by electrophoresis on an 11% SDS-polyacrylamide gel and were visualized by autoradiography.

Vaccination of Mice With VEE Virus Replicons

Groups of 10 BALB/c or C57BL/6 mice per experiment were subcutaneously injected at the base of the neck with 2 x 10^6 focus-forming units of VRPs encoding the Ebola virus genes. As controls, mice were also

injected with either a control VRP encoding the Lassa nucleoprotein (NP) or with PBS. For booster inoculations, animals received identical injections at 1 month intervals. Data are recorded as the combined results of 2 or 3 separate experiments.

Ebola Infection of Mice

One month after the final booster inoculation, mice were transferred to a BSL-4 containment area and challenged by intraperitoneal (ip) inoculation of 10 plaque-forming units (pfu) of mouse-adapted Ebola virus (approximately 300 times the dose lethal for 50% of adult mice). The mice were observed daily, and morbidity and mortality were recorded. Animals surviving at day 21 post-infection were injected again with the same dose of virus and observed for another 21 days.

In some experiments, 4 or 5 mice from vaccinated and control groups were anesthetized and exsanguinated on day 4 (BALB/c mice) or day 5 (C57BL/6 mice) following the initial viral challenge. The viral titers in individual sera were determined by plaque assay.

Passive Transfer Of Immune Sera to Naive Mice.

Donor sera were obtained 28 days after the third inoculation with 2 x 10^6 focus-forming units of VRPs encoding the indicated Ebola virus gene, the control Lassa NP gene, or from unvaccinated control mice. One mL of pooled donor sera was administered intraperitoneally (ip) to naive, syngeneic mice 24 h prior to intraperitoneal challenge with 10 pfu of mouse-adapted Ebola virus.

Vaccination and Challenge of Guinea Pigs.

EboGPVRP or EboNPVRP (1×10^7 focus-forming units in 0.5ml PBS) were administered subcutaneously to inbred strain 2 or strain 13 guinea pigs (300-400g). Groups of five guinea pigs were inoculated on days 0 and 28 at one (strain 2) or two (strain 13) dorsal

sites. Strain 13 guinea pigs were also boosted on day 126. One group of Strain 13 guinea pigs was vaccinated with both the GP and NP constructs. Blood samples were obtained after vaccination and after viral challenge. Guinea pigs were challenged on day 56 (strain 2) or day 160 (strain 13) by subcutaneous administration of 1000 LD_{50} (1×10^4 PFU) of guinea pig-adapted Ebola virus. Animals were observed daily for 60 days, and morbidity (determined as changes in behavior, appearance, and weight) and survival were recorded. Blood samples were taken on the days indicated after challenge and viremia levels were determined by plaque assay.

<u>Virus titration and neutralization assay</u>. Viral stocks were serially diluted in growth medium, adsorbed onto confluent Vero E6 cells in 6- or 12-well dishes, incubated for 1 hour at 37°C, and covered with an agarose overlay (Moe, J. *et al.* (1981) *J. Clin. Microbiol.* 13:791-793). A second overlay containing 5% neutral red solution in PBS or agarose was added 6 days later, and plaques were counted the following day. Pooled pre-challenge serum samples from some of the immunized groups were tested for the presence of Ebola-neutralizing antibodies by plaque reduction neutralization assay. Aliquots of Ebola virus in growth medium were mixed with serial dilutions of test serum, or with normal serum, or medium only, incubated at 37°C for 1 h, and used to infect Vero E6 cells. Plaques were counted 1 week later.

<u>Cytotoxic T cell assays</u>. BALB/c and C57BL/6 mice were inoculated with VRPs encoding Ebola virus NP or VP24 or the control Lassa NP protein. Mice were euthanized at various times after the last inoculation and their spleens removed. The spleens were gently ruptured to generate single cell suspensions. Spleen cells (1×10^6/ ml) were cultured *in vitro* for 2 days in the presence of 10-25 μM of peptides synthesized

from Ebola virus NP or VP24 amino acid sequences, and then for an additional 5 days in the presence of peptide and 10% supernatant from concanavalin A-stimulated syngeneic spleen cells. Synthetic peptides were made from Ebola virus amino acid sequences predicted by a computer algorithm (HLA Peptide Binding Predictions, Parker, K. C., et al. (1994) *J. Immunol.* **152**:163) to have a likelihood of meeting the MHC class I binding requirements of the BALB/c ($H\text{-}2^d$) and C57BL/6 ($H\text{-}2^b$) haplotypes. Only 2 of 8 peptides predicted by the algorithm and tested to date have been identified as containing CTL epitopes. After *in vitro* restimulation, the spleen cells were tested in a standard ^{51}chromium-release assay well known in the art (see, for example, Hart *et al.* (1991) *Proc. Natl. Acad. Sci. USA* **88**: 9449-9452). Percent specific lysis of peptide-coated, MHC-matched or mismatched target cells was calculated as:

$$\frac{\text{Experimental cpm- Spontaneous cpm} \times 100}{\text{Maximum cpm-Spontaneous cpm}}$$

Spontaneous cpm are the number of counts released from target cells incubated in medium. Maximum cpm are obtained by lysing target cells with 1% Triton X-100. Experimental cpm are the counts from wells in which target cells are incubated with varying numbers of effector (CTL) cells. Target cells tested were L5178Y lymphoma or P815 mastocytoma cells (MHC matched to the $H2^d$ BALB/c mice and EL4 lymphoma cells (MHC matched to the $H2^b$ C57BL/6 mice). The effector:target (E:T) ratios tested were 25:1, 12:1, 6:1 and 3:1.

EXAMPLE 1

Survival Of Mice Inoculated With VRPs Encoding Ebola Proteins. Mice were inoculated two or three times at 1 month intervals with 2×10^6 focus-forming

units of VRPs encoding individual Ebola virus genes, or Lassa virus NP as a control, or with phosphate buffered saline (PBS). Mice were challenged with 10 pfu of mouse-adapted Ebola virus one month after the final immunization. The mice were observed daily, and morbidity and mortality data are shown in Table 1A for BALB/c mice and Table 1B for C57BL/6 mice. The viral titers in individual sera of some mice on day 4 (BALB/c mice) or day 5 (C57BL/6 mice) following the initial viral challenge were determined by plaque assay.

Table 1. Survival Of Mice Inoculated With VRPs Encoding Ebola Proteins

A. BALB/c Mice

VRP	#Injections	S/T[1] (%)	MDD[2]	V/T[3]	Viremia[4]
EboNP	3	30/30(100%)	5/5	5.2	
	2	19/20 (95%)	7	5/5	4.6
EboGP	3	15/29 (52%)	8	1/5	6.6
	2	14/20 (70%)	7	3/5	3.1
EboVP24	3	27/30 (90%)	8	5/5	5.2
	2	19/20 (95%)	6	4/4	4.8
EboVP30	3	17/20 (85%)	7	5/5	6.2
	2	11/20 (55%)	7	5/5	6.5
EboVP35	3	5/19 (26%)	7	5/5	6.9
	2	4/20 (20%)	7	5/5	6.5
EboVP40	3	14/20 (70%)	8	5/5	4.6
	2	17/20 (85%)	7	5/5	5.6
LassaNP	3	0/29 (0%)	7	5/5	8.0
	2	0/20 (0%)	7	5/5	8.4

none (PBS)	3	1/30 (3%)	6	5/5	8.3
	2	0/20 (0%)	6	5/5	8.7

B. C57BL/6 Mice

VRP	#Injections	S/T[1] (%)	MDD[2]	V/T[3]	Viremia[4]
EboNP	3	15/20 (75%)	8	5/5	4.1
	2	8/10 (80%)	9	ND[5]	ND
EboGP	3	19/20 (95%)	10	0/5	--
	2	10/10 (100%)	–	ND	ND
EboVP24	3	0/20 (0%)	7	5/5	8.6
EboVP30	3	2/20 (10%)	8	5/5	7.7
EboVP35	3	14/20 (70%)	8	5/5	4.5
EboVP40	3	1/20 (5%)	7	4/4	7.8
LassaNP	3	1/20 (5%)	7	4/4	8.6
	2	0/10 (0%)	7	ND	ND
none(PBS)	3	3/20 (15%)	7	5/5	8.6
	2	0/10 (0%)	7	ND	ND

[1]S/T, Survivors/total challenged.

[2]MDD, Mean day to death

[3]V/T, Number of mice with viremia/total number tested.

[4]Geometric mean of Log_{10} viremia titers in PFU/mL. Standard errors for all groups were 1.5 or less, except for the group of BALB/c mice given 2 inoculations of EboGP, which was 2.2.

[5]ND, not determined.

EXAMPLE 2

VP24-Immunized BALB/c Mice Survive A High-Dose Challenge With Ebola virus.

BALB/c mice were inoculated two times with 2×10^6 focus-forming units of EboVP24VRP. Mice were challenged with either 1×10^3 pfu or 1×10^5 pfu of mouse-adapted Ebola virus 1 month after the second inoculation. Morbidity and mortality data for these mice are shown in Table 2.

Table 2. VP24-Immunized BALB/c Mice Survive A High-Dose Challenge With Ebola virus

Replicon	Challenge Dose	Survivors/Total
EboVP24	1×10^3 pfu (3×10^4 LD_{50})	5/5
EboVP24	1×10^5 pfu (3×10^6 LD_{50})	5/5
None	1×10^3 pfu (3×10^4 LD_{50})	0/4
None	1×10^5 pfu (3×10^6 LD_{50})	0/3

EXAMPLE 3

Passive Transfer Of Immune Sera Can Protect Naive Mice From A Lethal Challenge Of Ebola Virus.

Donor sera were obtained 28 days after the third inoculation with 2×10^6 focus-forming units of VRPs encoding the indicated Ebola virus gene, the control Lassa NP gene, or from unvaccinated control mice. One mL of pooled donor sera was administered

intraperitoneally (ip) to naive, syngeneic mice 24 h prior to intraperitoneal challenge with 10 pfu of mouse-adapted Ebola virus.

Table 3. Passive Transfer of Immune Sera Can Protect Unvaccinated Mice from a Lethal Challenge of Ebola Virus

A. BALB/c Mice

Specificity of Donor Sera	Survivors /Total	Mean Day of Death
Ebola GP	15/20	8
Ebola NP	1/20	7
Ebola VP24	0/20	6
Ebola VP30	0/20	7
Ebola VP35	ND[1]	ND
Ebola VP40	0/20	6
Lassa NP	0/20	7
Normal mouse sera	0/20	6

B. C57BL/6 Mice

Specificity of Donor Sera	Survivors /Total	Mean Day of Death
Ebola GP	17/20	7
Ebola NP	0/20	7
Ebola VP24	ND	ND
Ebola VP30	ND	ND
Ebola VP35	0/20	7
Ebola VP40	ND	ND
Lassa NP	0/20	7
Normal mouse sera	0/20	7

[1]ND, not determined

EXAMPLE 4

Immunogenicity and Efficacy of VRepEboGP and VRepEboNP in Guinea Pigs.

EboGPVRP or EboNPVRP ($1x10^7$ IU in 0.5ml PBS) were administered subcutaneously to inbred strain 2 or strain 13 guinea pigs (300-400g). Groups of five guinea pigs were inoculated on days 0 and 28 at one (strain 2) or two (strain 13) dorsal sites. Strain 13 guinea pigs were also boosted on day 126. One group of Strain 13 guinea pigs was vaccinated with both the GP and NP constructs. Blood samples were obtained after vaccination and after viral challenge.

Sera from vaccinated animals were assayed for antibodies to Ebola by plaque-reduction neutralization, and ELISA. Vaccination with VRepEboGP or NP induced high titers of antibodies to the Ebola proteins (Table 4) in both guinea pig strains. Neutralizing antibody responses were only detected in animals vaccinated with the GP construct (Table 4).

Guinea pigs were challenged on day 56 (strain 2) or day 160 (strain 13) by subcutaneous administration of 1000 LD_{50} (10^4 PFU) of guinea pig-adapted Ebola virus. Animals were observed daily for 60 days, and morbidity (determined as changes in behavior, appearance, and weight) and survival were recorded. Blood samples were taken on the days indicated after challenge and viremia levels were determined by plaque assay. Strain 13 guinea pigs vaccinated with the GP construct, alone or in combination with NP, survived lethal Ebola challenge (Table 4). Likewise, vaccination of strain 2 inbred guinea pigs with the GP construct protected 3/5 animals against death from lethal Ebola challenge, and significantly prolonged the mean day of death (MDD) in one of the two animals that died (Table 4). Vaccination with NP alone did not protect either guinea pig strain.

Table 4. Immunogenicity and efficacy of VRepEboGP and VRepEboNP in guinea pigs

A. Strain 2 guinea pigs

VRP	ELISA[a]	$PRNT_{50}$	Survivors/ total (MDD[b])		Viremia[c] d7	Viremia[c] d14
GP	4.1	30	3/5	(13+2.8)	2.3	1.8
NP	3.9	<10	0/5	(9.2+1.1)	3.0	--
Mock	<1.5	<10	0/5	(8.8+0.5)	3.9	--

B. Strain 13 guinea pigs

VRP	ELISA[a]	$PRNT_{50}$	Survivors/ total (MDD[b])	Viremia[c] d7	Viremia[c] d14
GP	4.0	140	5/5	<2.0	<2.0
GP/NP	3.8	70	5/5	<2.0	<2.0
NP	2.8	<10	1/5(8.3+2.2)	4.6	--
Lassa NP	<1.5	<10	2/5(8.3+0.6)	4.8	--

[a]Data are expressed as geometric mean titers, $\log_{10}$.

[b]MDD, mean day to death

[c]Geometric mean of $\log_{10}$ viremia titers in PFU/mL. Standard errors for all groups were 0.9 or less.

EXAMPLE 5

Induction of murine CTL responses to Ebola virus NP and Ebola virus VP24 proteins.

BALB/c and C57BL/6 mice were inoculated with VRPs encoding Ebola virus NP or VP24. Mice were euthanized at various times after the last inoculation and their spleens removed. Spleen cells (1×10^6/ ml) were cultured *in vitro* for 2 days in the presence of 10 to 25 μM of peptides, and then for an additional 5 days in the presence of peptide and 10% supernatant from concanavalin A-stimulated syngeneic spleen cells. After *in vitro* restimulation, the spleen cells were tested in a standard ^{51}chromium-release assay. Percent specific lysis of peptide-coated, MHC-matched or mismatched target cells was calculated as:

$$\frac{\text{Experimental cpm} - \text{Spontaneous cpm}}{\text{Maximum cpm} - \text{Spontaneous cpm}} \times 100$$

In the experiments shown, spontaneous release did not exceed 15%.

Table 5. Induction of murine CTL responses to Ebola virus NP and Ebola virus VP24 proteins.

			% Specific Lysis E:T ratio
Mice,VRP[1]	Peptide[2]	Cell[3]	25
BALB/c,VP24	None	P815	55
BALB/c,VP24	SEQ ID NO:25	P815	93
C57BL/6,EboNP	None	EL4	2
C57BL/6,EboNP[4]	SEQ ID NO:24	EL4	70
C57BL/6,EboNP	Lassa NP	EL4	2
C57BL/6,LassaNP	None	L5178Y	1
C57BL/6,LassaNP	SEQ ID NO:24	L5178Y	0
C57BL/6,LassaNP	None	EL4	2
C57BL/6,LassaNP	SEQ ID NO:24	EL4	6

[1] Indicates the mouse strain used and the VRP used as the *in vivo* immunogen. *In vitro* restimulation was performed using SEQ ID NO:24 peptide for BALB/c mice and SEQ ID NO:23 for all C57BL/6 mice shown.

[2] Indicates the peptide used to coat the target cells for the chromium release assay.

[3] Target cells are MHC-matched to the effector cells, except for the L5178Y cells that are C57BL/6 mismatched.

[4] High levels of specific lysis (>40%) were also observed using E:T ratios of 12, 6, 3, or 1:1.

RESULTS AND DISCUSSION

Ebola Zaire 1976 (Mayinga) virus causes acute hemorrhagic fever characterized by high mortality. There are no current vaccines or effective therapeutic measures to protect individuals who are exposed to this virus. In addition, it is not known which genes

are essential for evoking protective immunity and should therefore be included in a vaccine designed for human use. In this study, the GP, NP, VP24, VP30, VP35, and VP40 virion protein genes of the Ebola Zaire 1976 (Mayinga) virus were cloned and inserted into a Venezuelan equine encephalitis (VEE) virus replicon vector (VRep) as shown in Figure 2A and 2B. These VReps were packaged as VEE replicon particles (VRPs) using the VEE virus structural proteins provided as helper RNAs, as shown in Figure 3. This enables expression of the Ebola virus proteins in host cells. The Ebola virus proteins produced from these constructs were characterized *in vitro* and were shown to react with polyclonal rabbit anti-Ebola virus antibodies bound to Protein A beads following SDS gel electrophoresis of immunoprecipitated proteins (Figure 4).

The Ebola virus genes were sequenced from the VEE replicon clones and are listed here as SEQ ID NO:1 (GP), 2 (NP), 3 (VP24), 4 (VP30), 5 (VP35), 6 (VP40), and 7 (VP30#2) as described below. The corresponding amino acid sequences of the Ebola proteins expressed from these replicons are listed as SEQ ID NO: 17, 18, 19, 20, 21, 22, and 23, respectively. Changes in the DNA sequence relative to the sequence published by Sanchez *et al.* (1993) are described relative to the nucleotide (nt) sequence number from GenBank (accession number L11365).

The sequence we obtained for Ebola virus GP (SEQ ID NO:1) differed from the GenBank sequence by a transition from A to G at nt 8023. This resulted in a change in the amino acid sequence from Ile to Val at position 662 (SEQ ID NO: 17).

The DNA sequence we obtained for Ebola virus NP (SEQ ID NO:2) differed from the GenBank sequence at the following 4 positions: insertion of a C residue between nt 973 and 974, deletion of a G residue at nt 979, transition from C to T at nt 1307, and a

transversion from A to C at nt 2745. These changes resulted in a change in the protein sequence from Arg to Glu at position 170 and a change from Leu to Phe at position 280 (SEQ ID NO: 18).

The Ebola virus VP24 (SEQ ID NO:3) gene differed from the GenBank sequence at 6 positions, resulting in 3 nonconservative changes in the amino acid sequence. The changes in the DNA sequence of VP24 consisted of a transversion from G to C at nt 10795, a transversion from C to G at nt 10796, a transversion from T to A at nt 10846, a transversion from A to T at nt 10847, a transversion from C to G at nt 11040, and a transversion from C to G at nt 11041. The changes in the amino acid sequence of VP24 consisted of a Cys to Ser change at position 151, a Leu to His change at position 168, and a Pro to Gly change at position 233 (SEQ ID NO: 19).

We have included 2 different sequences for the Ebola virus VP30 gene (SEQ ID NOS:4 and SEQ ID NO:7). Both of these sequences differ from the GenBank sequence by the insertion of an A residue in the upstream noncoding sequence between nt 8469 and 8470 and an insertion of a T residue between nt 9275 and 9276 that results in a change in the open reading frame of VP30 and VP30#2 after position 255 (SEQ ID NOS:20 and SEQ ID NO:23). As a result, the C-terminus of the VP30 protein differs significantly from that previously reported. In addition to these 2 changes, the VP30#2 gene in SEQ ID NO:23 contains a conservative transition from T to C at nt 9217. Because the primers originally used to clone the VP30 gene into the replicon were designed based on the GenBank sequence, the first clone that we constructed (SEQ ID NO:4) did not contain what we believe to be the authentic C-terminus of the protein. Therefore, in the absence of the VP30 stop codon, the C-terminal codon was replaced with 37 amino acids derived from the vector sequence. The resulting VP30 construct

therefore differed from the GenBank sequence in that it contained 32 amino acids of VP30 sequence (positions 256 to 287, SEQ ID NO:20) and 37 amino acids of irrelevant sequence (positions 288 to 324, SEQ ID NO:20) in the place of the C-terminal 5 amino acids reported in GenBank. However, inclusion of 37 amino acids of vector sequence in place of the C-terminal amino acid (Pro, SEQ ID NO:23) did not inhibit the ability of the protein to serve as a protective antigen in BALB/c mice. We are currently examining the ability of the new VEE replicon construct (SEQ ID NO:7), which we believe contains the authentic C-terminus of VP30 (VP30#2, SEQ ID NO:23), to protect mice against a lethal Ebola challenge.

The DNA sequence for Ebola virus VP35 (SEQ ID NO:5) differed from the GenBank sequence by a transition from T to C at nt 4006, a transition from T to C at nt 4025, and an insertion of a T residue between nt 4102 and 4103. These sequence changes resulted in a change from a Ser to a Pro at position 293 and a change from Phe to Ser at position 299 (SEQ ID NO:21). The insertion of the T residue resulted in a change in the open reading frame of VP35 from that previously reported by Sanchez *et al.* (1993) following amino acid number 324. As a result, Ebola virus VP35 encodes for a protein of 340 amino acids, where amino acids 325 to 340 (SEQ ID NO:21) differ from and replace the C-terminal 27 amino acids of the previously published sequence.

Sequencing of VP30 and VP35 was also performed on RT/PCR products from RNA derived from cells that were infected with Ebola virus 1976, Ebola virus 1995 or the mouse-adapted Ebola virus. The changes noted above for the VRep constructs were also found in these Ebola viruses. Thus, we believe that these changes are real events and not artifacts of cloning.

The Ebola virus VP40 differed from the GenBank sequence by a transversion from a C to G at nt 4451

and a transition from a G to A at nt 5081. These sequence changes did not alter the protein sequence of VP40 (SEQ ID NO:22) from that of the published sequence.

To evaluate the protective efficacy of individual Ebola virus proteins and to determine whether the major histocompatibility (MHC) genes influence the immune response to Ebola virus antigens, two MHC-incompatible strains of mice were vaccinated with VRPs expressing an Ebola protein. As controls for these experiments, some mice were injected with VRPs expressing the nucleoprotein of Lassa virus or were injected with phosphate-buffered saline (PBS). Following Ebola virus challenge, the mice were monitored for morbidity and mortality, and the results are shown in Table 1.

The GP, NP, VP24, VP30, and VP40 proteins of Ebola virus generated either full or partial protection in BALB/c mice, and may therefore be beneficial components of a vaccine designed for human use. Vaccination with VRPs encoding the NP protein afforded the best protection. In this case, 100% of the mice were protected after three inoculations and 95% of the mice were protected after two inoculations. The VRP encoding VP24 also protected 90% to 95% of BALB/c mice against Ebola virus challenge. In separate experiments (Table 2), two or three inoculations with VRPs encoding the VP24 protein protected BALB/c mice from a high dose (1 x 10^5 plaque-forming units (3 x 10^6 LD50)) of mouse-adapted Ebola virus.

Vaccination with VRPs encoding GP protected 52-70% of BALB/c mice. The lack of protection was not due to a failure to respond to the VRP encoding GP, as all mice had detectable Ebola virus-specific serum antibodies after vaccination.

Some protective efficacy was also observed in BALB/c mice vaccinated two or three times with VRPs expressing the VP30 protein (55% and 85%,

respectively),or the VP40 protein (70% and 80%, respectively). The VP35 protein was not efficacious in the BALB/c mouse model, as only 20% and 26% of the mice were protected after either two or three doses, respectively.

Geometric mean titers of viremia were markedly reduced in BALB/c mice vaccinated with VRPs encoding Ebola virus proteins after challenge with Ebola virus, indicating an ability of the induced immune responses to reduce virus replication (Table 1A). In this study, immune responses to the GP protein were able to clear the virus to undetectable levels within 4 days after challenge in some mice.

When the same replicons were examined for their ability to protect C57BL/6 mice from a lethal challenge of Ebola virus, only the GP, NP, and VP35 proteins were efficacious (Table 1B). The best protection, 95% to 100%, was observed in C57BL/6 mice inoculated with VRPs encoding the GP protein. Vaccination with VRPs expressing NP protected 75% to 80% of the mice from lethal disease. In contrast to what was observed in the BALB/c mice, the VP35 protein was the only VP protein able to significantly protect the C57BL/6 mice. In this case, 3 inoculations with VRPs encoding VP35 protected 70% of the mice from Ebola virus challenge. The reason behind the differences in protection in the two mouse strains is not known but is believed to be due to the ability of the immunogens to sufficiently stimulate the cellular immune system. As with the BALB/c mice, the effects of the induced immune responses were also observed in reduced viremias and, occasionally, in a prolonged time to death of C57BL/6 mice.

VRPs expressing Ebola virus GP or NP were also evaluated for protective efficacy in a guinea pig model. Sera from vaccinated animals were assayed for antibodies to Ebola by western blotting, IFA, plaque-reduction neutralization, and ELISA. Vaccination with

either VRP (GP or NP) induced high titers of antibodies to the Ebola proteins (Table 4) in both guinea pig strains. Neutralizing antibody responses were only detected in animals vaccinated with the VRP expressing GP (Table 4).

Vaccination of strain 2 inbred guinea pigs with the GP construct protected 3/5 animals against death from lethal Ebola challenge, and significantly prolonged the mean day of death in one of the two animals that died (Table 4). All of the strain 13 guinea pigs vaccinated with the GP construct, alone or in combination with NP, survived lethal Ebola challenge (Table 4). Vaccination with NP alone did not protect either guinea pig strain from challenge with the guinea pig-adapted Ebola virus.

To identify the immune mechanisms that mediate protection against Ebola virus and to determine whether antibodies are sufficient to protect against lethal disease, passive transfer studies were performed. One mL of immune sera, obtained from mice previously vaccinated with one of the Ebola virus VRPs, was passively administered to unvaccinated mice 24 hours before challenge with a lethal dose of mouse-adapted Ebola virus. Antibodies to GP, but not to NP or the VP proteins, protected mice from an Ebola virus challenge (Table 3). Antibodies to GP protected 75% of the BALB/c mice and 85% of the C57BL/6 mice from death. When the donor sera were examined for their ability to neutralize Ebola virus in a plaque-reduction neutralization assay, a 1:20 to 1:40 dilution of the GP-specific antisera reduced the number of viral plaque-forming units by at least 50% (data not shown). In contrast, antisera to the NP and VP proteins did not neutralize Ebola virus at a 1:20 or 1:40 dilution. These results are consistent with the finding that GP is the only viral protein found on the surface of Ebola virus, and is likely to induce virus-neutralizing antibodies.

Since the NP and VP proteins of Ebola virus are internal virion proteins to which antibodies are not sufficient for protection, it is likely that cytotoxic T lymphocytes (CTLs) are also important for protection against Ebola virus. Initial studies aimed at identifying cellular immune responses to individual Ebola virus proteins expressed from VRPs identified CTL responses to the VP24 and NP proteins (Table 5). One CTL epitope that we identified for the Ebola virus NP is recognized by C57BL/6 (H-2^b) mice, and has an amino acid sequence of, or contained within, the following 11 amino acids: VYQVNNLEEIC (SEQ ID NO:24). Vaccination with EboNPVRP and *in vitro* restimulation of spleen cells with this peptide consistently induces strong CTL responses in C57BL/6 (H-2^b) mice. *In vivo* vaccination to Ebola virus NP is required to detect the CTL activity, as evidenced by the failure of cells from C57BL/6 mice vaccinated with Lassa NP to develop lytic activity to peptide (SEQ ID NO:24) after *in vitro* restimulation with it. Specific lysis has been observed using very low effector:target ratios (<2:1). This CTL epitope is H-2^b restricted in that it is not recognized by BALB/c (H-2^d) cells treated the same way (data not shown), and H-2^b effector cells will not lyse MHC-mismatched target cells coated with this peptide.

A CTL epitope in the VP24 protein was also identified. It is recognized by BALB/c (H-2^d) mice, and has an amino acid sequence of, or contained within, the following 23 amino acids: LKFINKLDALLVVNYNGLLSSIF (SEQ ID NO:25). In the data shown in Table 5, high (>90%) specific lysis of P815 target cells coated with this peptide was observed. The background lysis of cells that were not peptide-coated was also high (>50%), which is probably due to the activity of natural killer cells. We are planning to repeat this experiment using the L5178Y target

cells, which are not susceptible to natural killer cells.

Future studies will focus on determining the fine specificities of these CTL responses and the essential amino acids that constitute these CTL epitopes. Additional studies to identify other CTL epitopes on Ebola virus GP, NP, VP24, VP30, VP35, and VP40 will be performed. To evaluate the role of these CTLs in protection against Ebola virus, lymphocytes will be restimulated *in vitro* with peptides containing the CTL epitopes, and adoptively transferred into unvaccinated mice prior to Ebola virus challenge. In addition, future studies will examine the CTL responses to the other Ebola virus proteins to better define the roles of the cell mediated immune responses involved in protection against Ebola virus infection.

What is claimed is:

1. A DNA fragment which encodes a GP Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:1, or a polynucleotide fragment comprising at least 15 nucleotides.

2. A DNA fragment which encodes a NP Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:2, or a polynucleotide fragment comprising at least 15 nucleotides.

3. A DNA fragment which encodes a VP24 Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:3, or a polynucleotide fragment comprising at least 15 nucleotides.

4. A DNA fragment which encodes a VP30 Ebola protein, said DNA fragment comprising the sequence specified in any of SEQ ID NO:4 and SEQ ID NO:7, or a polynucleotide fragment comprising at least 15 nucleotides.

5. A DNA fragment which encodes a VP35 Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:5, or a polynucleotide fragment comprising at least 15 nucleotides.

6. A DNA fragment which encodes a VP40 Ebola protein, said DNA fragment comprising the sequence specified in SEQ ID NO:6, or a polynucleotide fragment comprising at least 15 nucleotides.

7. A DNA fragment which encodes a GP Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:17 or a conservative substitution thereof.

8. A DNA fragment which encodes a NP Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:18 or a conservative substitution thereof.

9. A DNA fragment which encodes a VP24 Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:19 or a conservative substitution thereof.

10. A DNA fragment which encodes a VP30 Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in any of SEQ ID NO:20 and SEQ ID NO:23 or a conservative substitution thereof.

11. A DNA fragment which encodes a VP35 Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:21 or a conservative substitution thereof.

12. A DNA fragment which encodes a VP40 Ebola protein said DNA fragment comprising a DNA sequence encoding at least 5 amino acids specified in SEQ ID NO:22 or a conservative substitution thereof.

13. A recombinant DNA construct comprising:
(i) a vector, and
(ii) at least one of the Ebola virus DNA fragments chosen from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 6 and 7 or a fragment thereof comprising at least 15 nucleotides.

14. A recombinant DNA construct comprising:
(i) a vector, and
(ii) at least one of the Ebola virus DNA fragments chosen from the group consisting of SEQ ID

NO: 17, 18, 19, 20, 21, 22, 23, 24 and 25 or a conservative substitution thereof.

15. The recombinant DNA construct of claim 13 wherein said DNA fragment induces a cytotoxic T lymphocyte response or antibody response.

16. The recombinant DNA construct of claim 14 wherein said DNA fragment induces a cytotoxic T lymphocyte response or antibody response.

17. A recombinant DNA construct according to claim 13 wherein said vector is an expression vector.

18. A recombinant DNA construct according to claim 13 wherein said vector is a prokaryotic vector.

19. A recombinant DNA construct according to claim 13 wherein said vector is a eukaryotic vector.

20. A recombinant DNA construct according to claim 14 wherein said vector is an expression vector.

21. A recombinant DNA construct according to claim 14 wherein said vector is a prokaryotic vector.

22. A recombinant DNA construct according to claim 14 wherein said vector is a eukaryotic vector.

23. The recombinant DNA construct of claim 17 wherein said vector is a VEE virus replicon vector.

24. The recombinant DNA construct of claim 20 wherein said vector is a VEE virus replicon vector.

25. The recombinant DNA construct according to claim 23 wherein said Ebola virus DNA fragments are from Ebola Zaire 1976.

26. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP24.

27. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP30.

28. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP35.

29. The recombinant DNA construct according to claim 25 wherein said construct is VRepEboVP40.

30. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboNP.

31. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboGP.

32. The recombinant DNA construct according to claim 25 wherein said construct is for VRepEboVP30(#2).

33. Self replicating RNA produced from a construct chosen from the group consisting of EboVP24ReP, EboVP30ReP, EboVP35ReP, EboVP40ReP, EboVPNPReP, EboVPGPReP, and EboVP30ReP(#2).

34. Infectious alphavirus particles produced from packaging the self replicating RNA of claim 33.

35. A pharmaceutical composition comprising infectious alphavirus particles according to claim 34 in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

36. A host cell transformed with a recombinant DNA construct according to claim 13.

37. A host cell transformed with a recombinant DNA construct according to claim 14.

38. A host cell according to claim 36 wherein said host cell is prokaryotic.

39. A host cell according to claim 36 wherein said host cell is eukaryotic.

40. A host cell according to claim 37 wherein said host cell is prokaryotic.

41. A host cell according to claim 37 wherein said host cell is eukaryotic.

42. A method for producing Ebola virus proteins comprising culturing the cells according to claim 36 under conditions such that said DNA fragment is expressed and said Ebola protein is produced.

43. A method for producing Ebola virus proteins comprising culturing the cells according to claim 37 under conditions such that said DNA fragment is expressed and said Ebola protein is produced.

44. A method for producing Ebola virus proteins comprising culturing the cells according to claim 38 under conditions such that said DNA fragment is expressed and said Ebola protein is produced.

45. A method for producing Ebola virus proteins comprising culturing the cells according to claim 39 under conditions such that said DNA fragment is expressed and said Ebola protein is produced.

46. An isolated and purified Ebola GP protein specified in SEQ ID NO:17 and conservative substitutions thereof, or an immunologically identifiable portion thereof.

47. An isolated and purified Ebola NP protein specified in SEQ ID NO:18 and conservative substitutions thereof or an immunologically identifiable portion thereof.

48. An isolated and purified Ebola VP24 protein specified in SEQ ID NO:19 and conservative substitutions thereof or an immunologically identifiable portion thereof.

49. An isolated and purified Ebola VP30 protein specified in any of SEQ ID NO:20 and SEQ ID NO:23 and conservative substitutions thereof or an immunologically identifiable portion thereof.

50. An isolated and purified Ebola VP35 protein specified in SEQ ID NO:21 and conservative substitutions thereof or an immunologically identifiable portion thereof.

51. An isolated and purified Ebola VP40 protein specified in SEQ ID NO:22 and conservative substitutions thereof or an immunologically identifiable portion thereof.

52. An antibody to a peptide encoded by the sequence specified in SEQ ID NO:17, 18, 19, 20, 21, 22, 23, 24, and 25.

53. A method for detecting Ebola virus infection comprising contacting a sample from a subject suspected of having Ebola virus infection with a antibody according to claim 52 and detecting the

presence or absence by detecting the presence or absence of a complex formed between the Ebola protein and antibodies specific therefor.

54. A method for detecting the presence or absence of Ebola virus GP RNA in a sample using the polymerase chain reaction using primers for Ebola GP nucleic acid sequence specified in SEQ ID NO:1 for GP.

55. An Ebola infection diagnostic kit comprising at least 12 consecutive nucleotides of SEQ ID NO:1 specific for the amplification of DNA or RNA of Ebola virus in a sample using the polymerase chain reaction and ancillary reagents suitable for use in such a reaction for detecting the presence or absence of Ebola virus DNA or RNA in a sample.

56. A vaccine for Ebola comprising alphavirus particles of claim 34.

57. A method for the diagnosis of Ebola virus infection comprising the steps of:

(i) contacting a sample from an individual suspected of having Ebola virus infection with an antibody to Ebola proteins according to claim 52; and

(ii) detecting the presence or absence of Ebola virus infection by detecting the presence or absence of a complex formed between Ebola proteins and antibodies specific therefor.

58. A pharmaceutical composition comprising the self replicating RNA of claim 33 in an effective immunogenic amount in a pharmaceutically acceptable carrier and/or adjuvant.

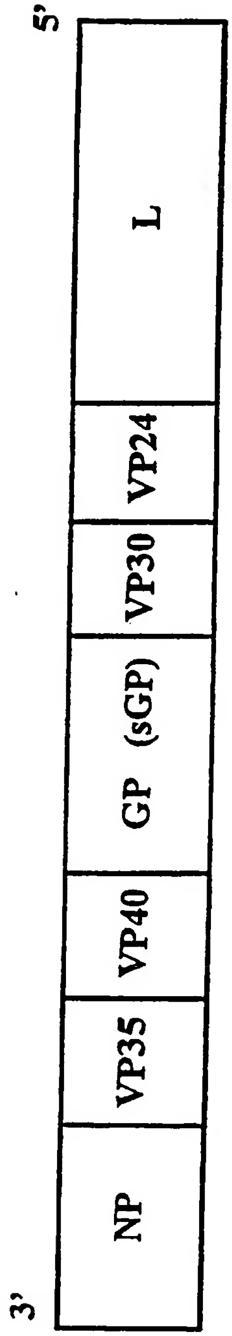
59. A pharmaceutical composition comprising one or more recombinant DNA constructs chosen from the group consisting of VRepEboVP24, VRepEboVP30, VRepEboVP35,

VRepEboVP40, VRepEboNP, VRepEboGP, and VRepEboVP30(#2), in a pharmaceutically acceptable amount, in a pharmaceutically accpetable carrier and/or adjuvant.

60. A pharmaceutical composition comprising comprising a peptide encoded by any of SEQ ID NO:24 and SEQ ID NO:25, in a pharmaceutically acceptable amount, in a pharmaceutically acceptable carrier and/or adjuvant.

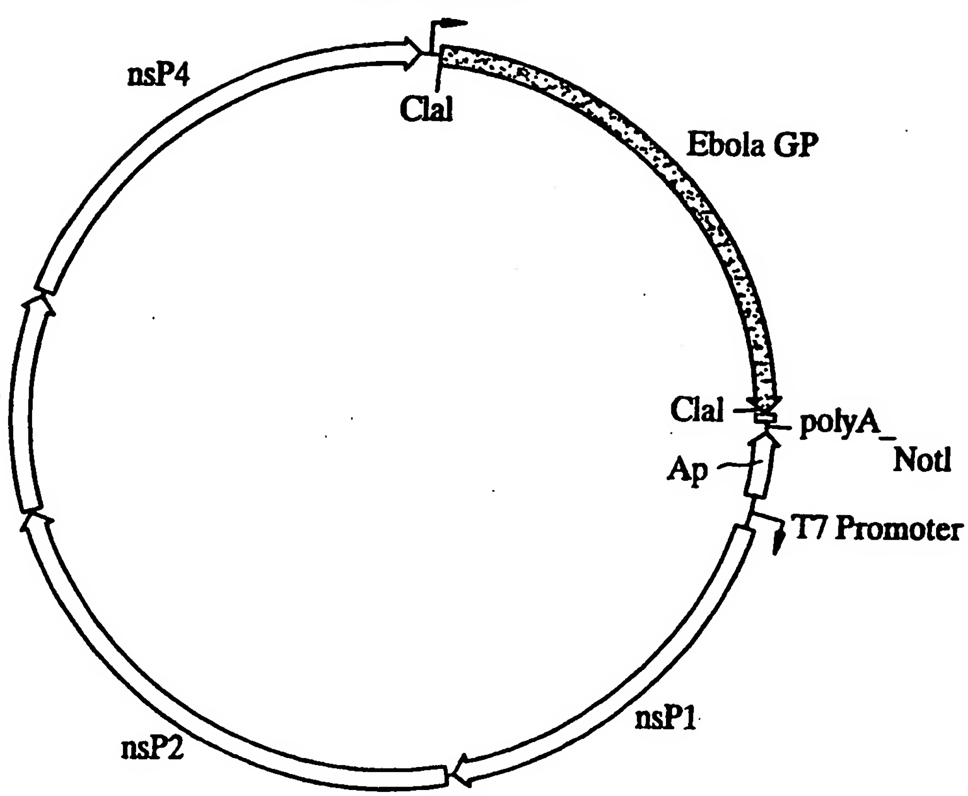
FIG. 1

Organization of the Ebola Virus Genome



NP	Major Nucleocapsid Protein
VP35	Phosphoprotein
VP40	Membrane-Associated Matrix Protein
GP	Transmembrane Glycoprotein
sGP	Secreted Glycoprotein
VP30	Ribonucleoprotein Associated (Minor)
VP24	Membrane-Associated Protein (Minor)
L	RNA-Dependent RNA Polymerase

FIG.2A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ClaI
polyA
NotI
Ap
T7 Promoter
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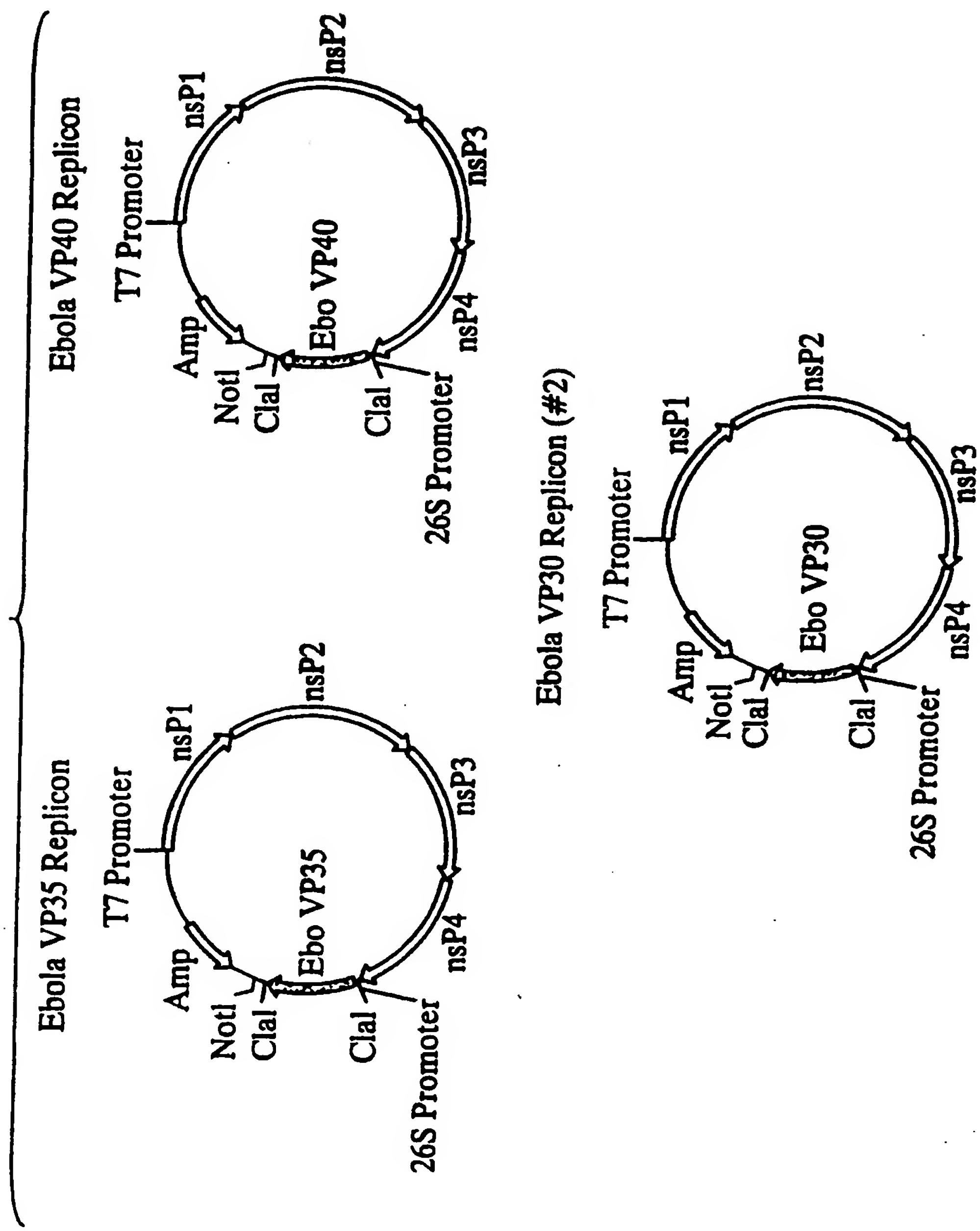
nsP2

FIG.2B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T7 Promoter
nsP1
nsP2
nsP3
nsP4
Ebo VP35
Amp
NotI
ClaI
ClaI
26S Promoter
Ebola VP30 Replicon (#2)
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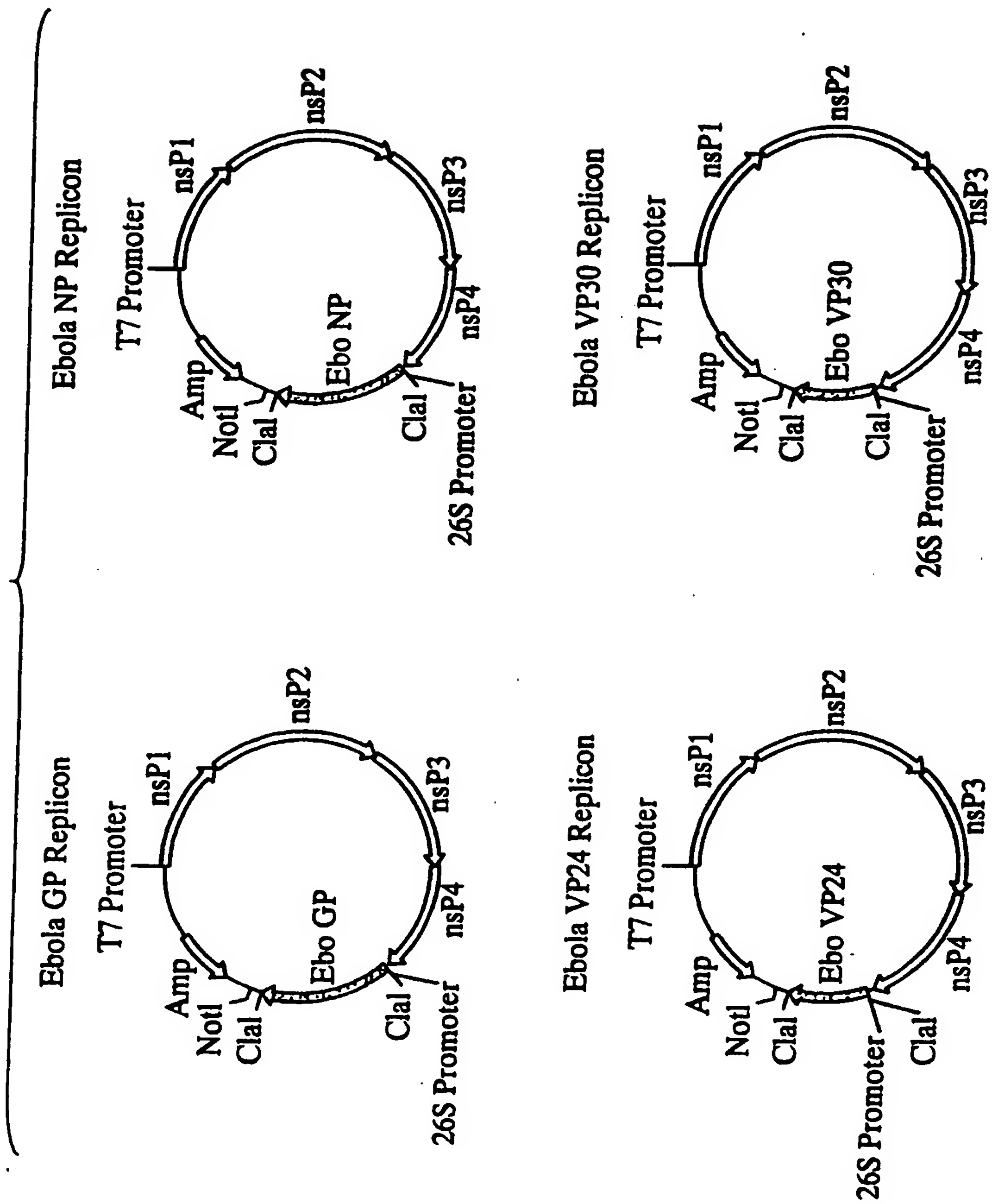
FIG.2C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nsP2
nsP3
nsP4
Ebo VP30
Amp
NotI
ClaI
ClaI
26S Promoter
Ebola GP Replicon
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NotI
ClaI
ClaI
26S Promoter
Ebola VP24 Replicon
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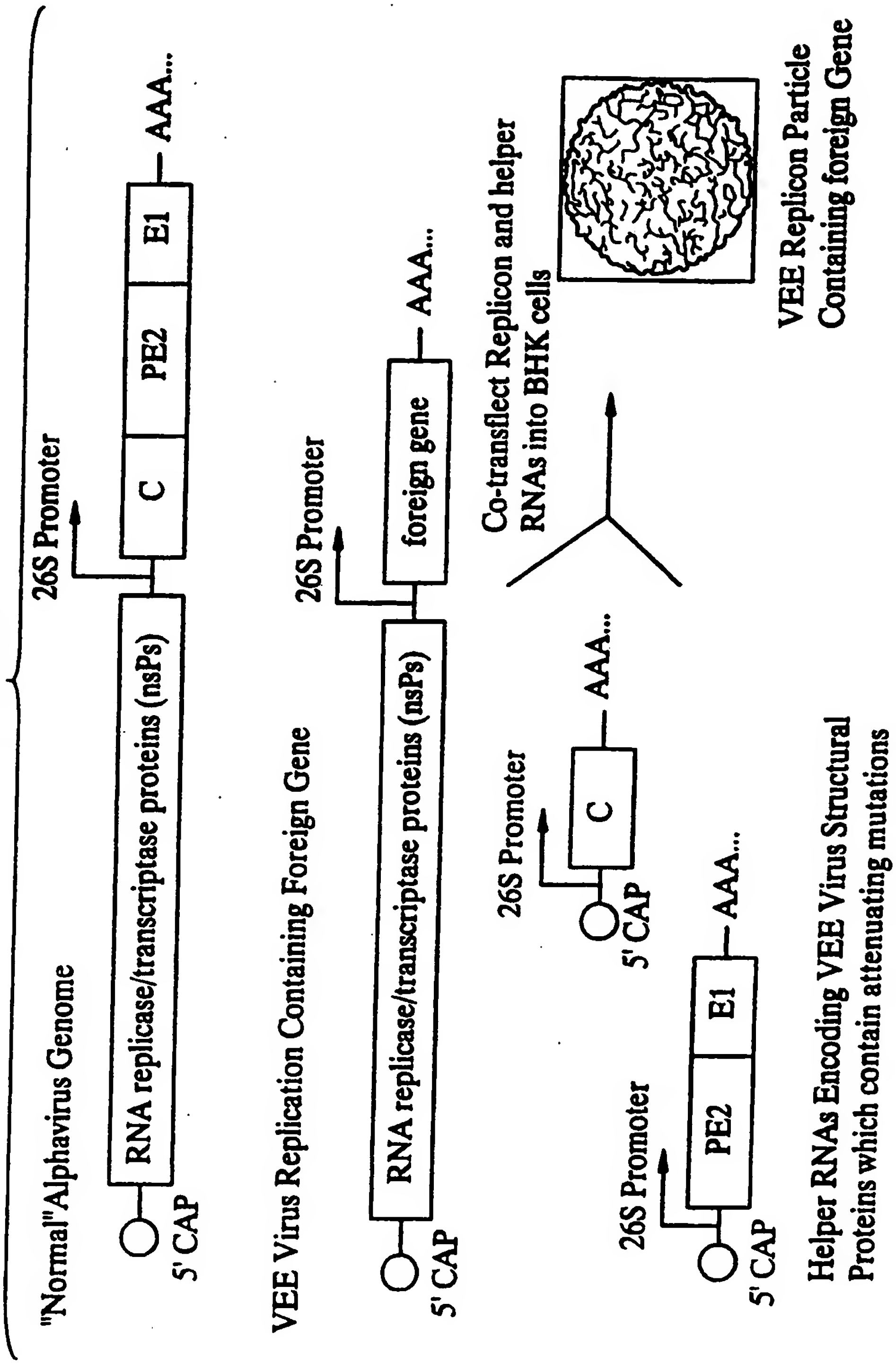
FIG.3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5' CAP
RNA replicase/transcriptase proteins (nsPs)
26S Promoter
C
PE2
E1
AAA...
VEE Virus Replication Containing Foreign Gene
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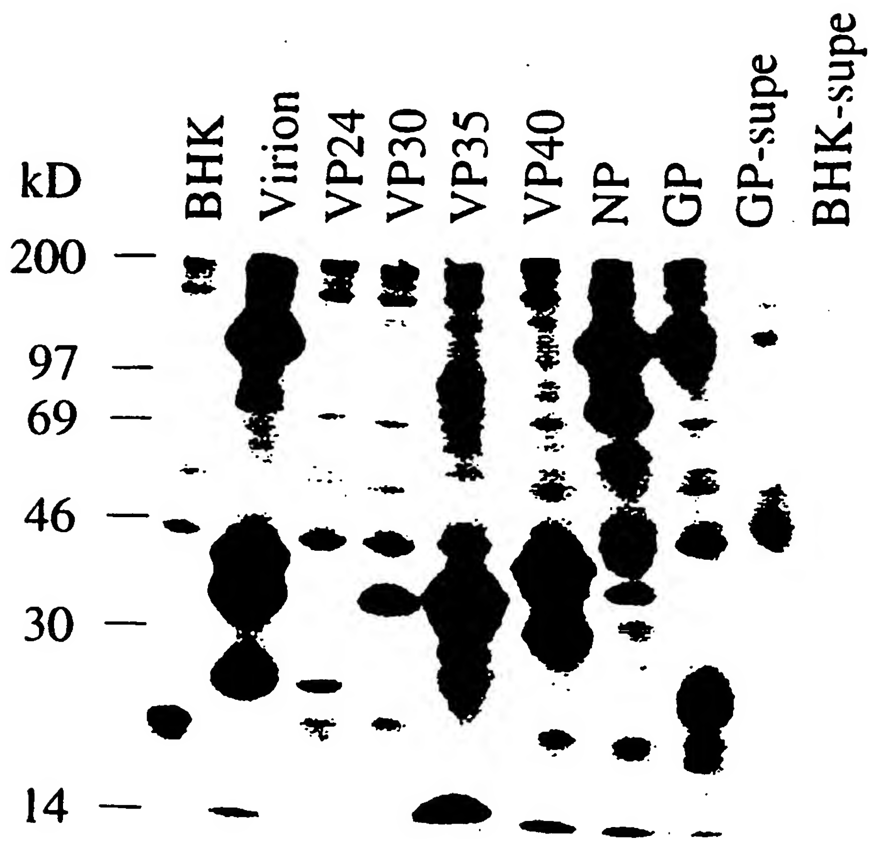
RNA replicase/transcriptase proteins (nsPs)
26S Promoter
foreign gene
AAA...
26S Promoter
5' CAP
PE2
E1
AAA...
26S Promoter
5' CAP
C
AAA...
Helper RNAs Encoding VEE Virus Structural
Proteins which contain attenuating mutations
Co-transflect Replicon and helper
RNAs into BHK cells
VEE Replicon Particle
Containing foreign Gene

FIG. 4

Eboli Protiens Expressed from VEE Replicons



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<110> United States Army Medical Research Institute of Infectious Diseases
Hart, Mary Katherine
Wilson, Julie A.
Pushko, Peter
Smith, Jonathan F.
Schmaljohn, Alan L.

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<140> PCT/US99/14311

<141> 1999-06-22

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caacaacact catcaccaag ataccggaga agagagtgcc   1480
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gagtcgcagg actgatcaca ggcgggagaa gaactcgaag   1560
agaagcaatt gtcaatgctc aacccaaatg caaccctaat   1600
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ctcttcaact gttcctgaga gccacaactg agctacgcac   1800
cttttcaatc ctcaaccgta aggcaattga tttcttgctg   1840
cagcgatggg gcggcacatg ccacattctg ggaccggact   1880
gctgtatcga accacatgat tggaccaaga acataacaga   1920
caaaattgat cagattattc atgattttgt tgataaaacc   1960
cttccggacc agggggacaa tgacaattgg tggacaggat   2000
ggagacaatg gataccggca ggtattggag ttacaggcgt   2040
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agcctcaaat caatgaaacc aggatttaat tatatggatt   2160
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actggagctt taaacatagc caatgtgatt ctaactcctt   2240
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<210> 2

<211> 2428

<212> DNA

<213> Ebola Zaire

<220>

<400> 2

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gcaggtctgt ccgttcaaca ggggattgtt cggcaaagag    160
tcatcccagt gtatcaagta aacaatcttg aagaaatttg    200
ccaacttatc atacaggcct ttgaagcagg tgttgatttt    240
caagagagtg cggacagttt ccttctgatg ctttgtcttc    280
atcatgcgta ccagggagat tacaaacttt tcttggaaag    320
tggcgcagtc aagtatttgg aagggcacgg gttccgtttt    360
gaagtcaaga agcgtgatgg agtgaagcga cttgaggaat    400
tgctgccagc agtatctagt ggaaaaaaca ttaagagaac    440
acttgctgcc atgccggaag aggagacaac tgaagctaat    480
gccggtcagt ttctctcctt tgcaagtcta ttccttccga    520
aattggtagt aggagaaaag gcttgccttg agaaggttca    560
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tatccaacag cttggcaatc agtaggacac atgatggtga    640
ttttccgttt gatgcgaaca aattttctga tcaaatttct    680
cctaatacac caagggatgc acatggttgc cgggcatgat    720
gccaacgatg ctgtgatttc aaattcagtg gctcaagctc    760
gtttttcagg cttattgatt gtcaaaacag tacttgatca    800
tatcctacaa aagacagaac gaggagttcg tctccatcct    840
cttgcaagga ccgccaaggt aaaaaatgag gtgaactcct    880
ttaaggctgc actcagctcc ctggccaagc atggagagta    920
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cactcggagt cgccacagca cacgggagta ccctcgcagg   1040
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gccactgagg ctgagaagca actccaacaa tatgcagagt   1120
ctcgcgaact tgaccatctt ggacttgatg atcaggaaaa   1160
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cagatgactt ggtcctattc gatctagacg aggacgacga   1520
ggacactaag ccagtgccta atagatcgac caagggtgga   1560
caacagaaga acagtcaaaa gggccagcat atagagggca   1600
gacagacaca atccaggcca attcaaaatg tcccaggccc   1640
tcacagaaca atccaccacg ccagtgcgcc actcacggac   1680
aatgacagaa gaaatgaacc ctccggctca accagccctc   1720
gcatgctgac accaattaac gaagaggcag acccactgga   1760
cgatgccgac gacgagacgt ctagccttcc gcccttggag   1800
tcagatgatg aagagcagga cagggacgga acttccaacc   1840
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gcacacccac tgtcgcccca ccggctcccg tatacagaga 1880
tcactctgaa aagaaagaac tcccgcaaga cgagcaacaa 1920
gatcaggacc acactcaaga ggccaggaac caggacagtg 1960
acaacaccca gtcagaacac tcttttgagg agatgtatcg 2000
ccacattcta agatcacagg ggccatttga tgctgttttg 2040
tattatcata tgatgaagga tgagcctgta gttttcagta 2080
ccagtgatgg caaagagtac acgtatccag actcccttga 2120
agaggaatat ccaccatggc tcactgaaaa agaggctatg 2160
aatgaagaga atagatttgt tacattggat ggtcaacaat 2200
tttattggcc ggtgatgaat cacaagaata aattcatggc 2240
aatcctgcaa catcatcagt gaatgagcat ggaacaatgg 2280
gatgattcaa ccgacaaata gctaacatta agtagtccag 2320
gaacgaaaac aggaagaatt tttgatgtct aaggtgtgaa 2360
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<210> 3

<211> 847

<212> DNA

<213> Ebola Zaire

<220>

<400> 3

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aacttcttag ttagccaaac tattcagggg tggaaggttt 160
attgggctgg tattgagttt gatgtgactc acaaaggaat 200
ggccctattg catagactga aaactaatga ctttgcccct 240
gcatggtcaa tgacaaggaa tctctttcct catttatttc 280
aaaatccgaa ttccacaatt gaatcaccgc tgtgggcatt 320
gagagtcatc cttgcagcag ggatacagga ccagctgatt 360
gaccagtctt tgattgaacc cttagcagga gcccttggtc 400
tgatctctga ttggctgcta acaaccaaca ctaaccattt 440
caacatgcga acacaacgtg tcaaggaaca attgagccta 480
aaaatgctgt cgttgattcg atccaatatt ctcaagttta 520
ttaacaaatt ggatgctcta catgtcgtga actacaacgg 560
attgttgagc agtattgaaa ttggaactca aaatcataca 600
atcatcataa ctcgaactaa catgggtttt ctggtggagc 640
tccaagaacc cgacaaatcg gcaatgaacc gcatgaagcc 680
tgggccggcg aaattttccc tccttcatga gtccacactg 720
aaagcattta cacaaggatc ctcgacacga atgcaaagtt 760
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<210> 4

<211> 973

<212> DNA

<213> Ebola Zaire

<220>

<400> 4

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atgagagagg acgcccacga gctgccagac agcattcaag    160
ggatggacac gaccaccatg ttcgagcacg atcatcatcc    200
agagagaatt atcgaggtga gtaccgtcaa tcaaggagcg    240
cctcacaagt gcgcgttcct actgtatttc ataagaagag    280
agttgaacca ttaacagttc ctccagcacc taaagacata    320
tgtccgacct tgaaaaaagg atttttgtgt gacagtagtt    360
tttgcaaaaa agatcaccag ttggagagtt taactgatag    400
ggaattactc ctactaatcg cccgtaagac ttgtggatca    440
gtagaacaac aattaaatat aactgcaccc aaggactcgc    480
gcttagcaaa tccaacggct gatgatttcc agcaagagga    520
aggtccaaaa attaccttgt tgacactgat caagacggca    560
gaacactggg cgagacaaga catcagaacc atagaggatt    600
caaaattaag agcattgttg actctatgtg ctgtgatgac    640
gaggaaattc tcaaaatccc agctgagtct tttatgtgag    680
acacacctaa ggcgcgaggg gcttgggcaa gatcaggcag    720
aacccgttct cgaagtatat caacgattac acagtgataa    760
aggaggcagt tttgaagctg cactatggca acaatgggac    800
ctacaatccc taattatgtt tatcactgca ttcttgaata    840
ttgctctcca gttaccgtgt gaaagttctg ctgtcgttgt    880
ttcagggtta agaacattgg ttcctcaatc agataatgag    920
gaagcttcaa ccaacccggg gacatgctca tggtctgatg    960
agggtacatc gat                                 973
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<210> 5

<211> 1148

<212> DNA

<213> Ebola Zaire

<220>

<400> 5

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agctaatgac cggaagaatt cctgtaagcg acatcttctg    160
tgatattgag aacaatccag gattatgcta cgcatcccaa    200
atgcaacaaa cgaagccaaa cccgaagacg cgcaacagtc    240
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aaacccaaac ggacccaatt tgcaatcata gttttgagga   280
ggtagtacaa acattggctt cattggctac tgttgtgcaa   320
caacaaacca tcgcatcaga atcattagaa caacgcatta   360
cgagtcttga gaatggtcta aagccagttt atgatatggc   400
aaaaacaatc tcctcattga acagggtttg tgctgagatg   440
gttgcaaaat atgatcttct ggtgatgaca accggtcggg   480
caacagcaac cgctgcggca actgaggctt attgggccga   520
acatggtcaa ccaccacctg gaccatcact ttatgaagaa   560
agtgcgattc ggggtaagat tgaatctaga gatgagaccg   600
tccctcaaag tgttagggag gcattcaaca atctaaacag   640
taccacttca ctaactgagg aaaatttgg gaaacctgac    680
atttcggcaa aggatttgag aaacattatg tatgatcact   720
tgcctggttt tggaactgct ttccaccaat tagtacaagt   760
gatttgtaaa ttgggaaaag atagcaactc attggacatc   800
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atgtgttttt cagcttcaag atggtaaaac acttggactc  1040
aaaatttgag ccaatctccc ttccctccga aagaggcgaa  1080
taatagcaga ggcttcaact gctgaactat agggtacgtt  1120
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<210> 6

<211> 1123

<212> DNA

<213> Ebola Zaire

<220>

<400> 6

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ccgatgacac catcgaccat gccagccaca caccaggcag   280
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tgactcaact acggccgcca tcatgcttgc ttcatacact   440
atcacccatt tcggcaaggc aaccaatcca cttgtcagag   480
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gctcctgcga attggaaacc aggctttcct ccaggagttc   560
gttcttccgc cagtccaact accccagtat ttcacctttg   600
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tgcaacatgg accgatgaca ctccaacagg atcaaatgga   680
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acaggattgt gacacgtgtc attctcctgc aagtcttcca 1040
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agttttatag aatcttctca gggatagtgc ataacatatc 1120
gat                                         1123
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<210> 7

<211> 1165

<212> DNA

<213> Ebola Zaire

<220>

<400> 7

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atgagagagg acgcccacga gctgccagac agcattcaag 160
ggatggacac gaccaccatg ttcgagcacg atcatcatcc 200
agagagaatt atcgaggtga gtaccgtcaa tcaaggagcg 240
cctcacaagt gcgcgttcct actgtatttc ataagaagag 280
agttgaacca ttaacagttc ctccagcacc taaagacata 320
tgtccgacct tgaaaaaagg atttttgtgt gacagtagtt 360
tttgcaaaaa agatcaccag ttggagagtt taactgatag 400
ggaattactc ctactaatcg cccgtaagac ttgtggatca 440
gtagaacaac aattaaatat aactgcaccc aaggactcgc 480
gcttagcaaa tccaacggct gatgatttcc agcaagagga 520
aggtccaaaa attaccttgt tgacactgat caagacggca 560
gaacactggg cgagacaaga catcagaacc atagaggatt 600
caaaattaag agcattgttg actctatgtg ctgtgatgac 640
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aacccgttct cgaagtatat caacgattac acagtgataa 760
aggaggcagt tttgaagctg cactatggca acaatggac 800
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cttctacttg atcacaatac tccgtatacc tatcatcata 1040
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tgccctaata tatgaagagg tatgatacaa ccctaacaga 1160
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<210> 8

<211> 30

<212> DNA

<213> artificial sequence

<220>

<223> /note= "forward primer for VP24"

<400> 8

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<210> 9

<211> 33

<212> DNA

<213> artificial sequence

<220>

<223> /note= "reverse primer for VP24"

<400> 9

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<210> 10

<211> 30

<212> DNA

<213> artificial sequence

<220>

<223> /note= "forward primer for VP30"

<400> 10

cccatcgatc agatctgcga accggtagag 30

<210> 11

<211> 31

<212> DNA

<213> artificial sequence

<220>

<223> /note= "reverse primer for VP30"

<400> 11

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<210> 12

<211> 33

<212> DNA

<213> artificial sequence

<220>

<223> /note= "forward primer for VP35"

<400> 12

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<210> 13

<211> 36

<212> DNA

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<210> 14

<211> 30

<212> DNA

<213> artificial sequence

<220>

<223> /note= "forward primer for VP40"

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<210> 15

<211> 33

<212> DNA

<213> artificial sequence

<220>

<223> /note= "reverse primer for VP40"

<400> 15

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<210> 16

<211> 30

<212> DNA

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<220>

<223> /note= "reverse primer for VP30#2"

<400> 16

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<210> 17

<211> 676

<212> PRT

<213> Ebola Zaire

<220>

<400> 17

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15 20
Leu Trp Val Ile Ile Leu Phe Gln Arg Thr
25 30
Phe Ser Ile Pro Leu Gly Val Ile His Asn
35 40
Ser Thr Leu Gln Val Ser Asp Val Asp Lys
45 50
Leu Val Cys Arg Asp Lys Leu Ser Ser Thr
55 60
Asn Gln Leu Arg Ser Val Gly Leu Asn Leu
65 70
Glu Gly Asn Gly Val Ala Thr Asp Val Pro
75 80
Ser Ala Thr Lys Arg Trp Gly Phe Arg Ser
85 90
Gly Val Pro Pro Lys Val Val Asn Tyr Glu
95 100
Ala Gly Glu Trp Ala Glu Asn Cys Tyr Asn
105 110
Leu Glu Ile Lys Lys Pro Asp Gly Ser Glu
115 120
Cys Leu Pro Ala Ala Pro Asp Gly Ile Arg
125 130
Gly Phe Pro Arg Cys Arg Tyr Val His Lys
135 140
Val Ser Gly Thr Gly Pro Cys Ala Gly Asp
145 150
Phe Ala Phe His Lys Glu Gly Ala Phe Phe
155 160
Leu Tyr Asp Arg Leu Ala Ser Thr Val Ile
165 170
Tyr Arg Gly Thr Thr Phe Ala Glu Gly Val
175 180
Val Ala Phe Leu Ile Leu Pro Gln Ala Lys
185 190
Lys Asp Phe Phe Ser Ser His Pro Leu Arg
195 200
Glu Pro Val Asn Ala Thr Glu Asp Pro Ser
205 210
Ser Gly Tyr Tyr Ser Thr Thr Ile Arg Tyr
215 220
Gln Ala Thr Gly Phe Gly Thr Asn Glu Thr
225 230

Glu Tyr Leu Phe Glu Val Asp Asn Leu Thr
235 240
Tyr Val Gln Leu Glu Ser Arg Phe Thr Pro
245 250
Gln Phe Leu Leu Gln Leu Asn Glu Thr Ile
255 260
Tyr Thr Ser Gly Lys Arg Ser Asn Thr Thr
265 270
Gly Lys Leu Ile Trp Lys Val Asn Pro Glu
275 280
Ile Asp Thr Thr Ile Gly Glu Trp Ala Phe
285 290
Trp Glu Thr Lys Lys Asn Leu Thr Arg Lys
295 300
Ile Arg Ser Glu Glu Leu Ser Phe Thr Val
305 310
Val Ser Asn Gly Ala Lys Asn Ile Ser Gly
315 320
Gln Ser Pro Ala Arg Thr Ser Ser Asp Pro
325 330
Gly Thr Asn Thr Thr Thr Glu Asp His Lys
335 340
Ile Met Ala Ser Glu Asn Ser Ser Ala Met
345 350
Val Gln Val His Ser Gln Gly Arg Glu Ala
355 360
Ala Val Ser His Leu Thr Thr Leu Ala Thr
365 370
Ile Ser Thr Ser Pro Gln Ser Leu Thr Thr
375 380
Lys Pro Gly Pro Asp Asn Ser Thr His Asn
385 390
Thr Pro Val Tyr Lys Leu Asp Ile Ser Glu
395 400
Ala Thr Gln Val Glu Gln His His Arg Arg
405 410
Thr Asp Asn Asp Ser Thr Ala Ser Asp Thr
415 420
Pro Ser Ala Thr Thr Ala Ala Gly Pro Pro
425 430
Lys Ala Glu Asn Thr Asn Thr Ser Lys Ser
435 440
Thr Asp Phe Leu Asp Pro Ala Thr Thr Thr
445 450
Ser Pro Gln Asn His Ser Glu Thr Ala Gly
455 460
Asn Asn Asn Thr His His Gln Asp Thr Gly
465 470
Glu Glu Ser Ala Ser Ser Gly Lys Leu Gly
475 480
Leu Ile Thr Asn Thr Ile Ala Gly Val Ala
485 490
Gly Leu Ile Thr Gly Gly Arg Arg Thr Arg
495 500

Arg Glu Ala Ile Val Asn Ala Gln Pro Lys
505 510
Cys Asn Pro Asn Leu His Tyr Trp Thr Thr
515 520
Gln Asp Glu Gly Ala Ala Ile Gly Leu Ala
525 530
Trp Ile Pro Tyr Phe Gly Pro Ala Ala Glu
535 540
Gly Ile Tyr Ile Glu Gly Leu Met His Asn
545 550
Gln Asp Gly Leu Ile Cys Gly Leu Arg Gln
555 560
Leu Ala Asn Glu Thr Thr Gln Ala Leu Gln
565 570
Leu Phe Leu Arg Ala Thr Thr Glu Leu Arg
575 580
Thr Phe Ser Ile Leu Asn Arg Lys Ala Ile
585 590
Asp Phe Leu Leu Gln Arg Trp Gly Gly Thr
595 600
Cys His Ile Leu Gly Pro Asp Cys Cys Ile
605 610
Glu Pro His Asp Trp Thr Lys Asn Ile Thr
615 620
Asp Lys Ile Asp Gln Ile Ile His Asp Phe
625 630
Val Asp Lys Thr Leu Pro Asp Gln Gly Asp
635 640
Asn Asp Asn Trp Trp Thr Gly Trp Arg Gln
645 650
Trp Ile Pro Ala Gly Ile Gly Val Thr Gly
655 660
Val Val Ile Ala Val Ile Ala Leu Phe Cys
665 670
Ile Cys Lys Phe Val Phe
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<210> 18

<211> 739

<212> PRT

<213> Ebola Zaire

<220>

<400> 18

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Ala Pro Ser Leu Thr Glu Ser Asp Met Asp
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Tyr His Lys Ile Leu Thr Ala Gly Leu Ser

25 30
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35 40
Ile Pro Val Tyr Gln Val Asn Asn Leu Glu
45 50
Glu Ile Cys Gln Leu Ile Ile Gln Ala Phe
55 60
Glu Ala Gly Val Asp Phe Gln Glu Ser Ala
65 70
Asp Ser Phe Leu Leu Met Leu Cys Leu His
75 80
His Ala Tyr Gln Gly Asp Tyr Lys Leu Phe
85 90
Leu Glu Ser Gly Ala Val Lys Tyr Leu Glu
95 100
Gly His Gly Phe Arg Phe Glu Val Lys Lys
105 110
Arg Asp Gly Val Lys Arg Leu Glu Glu Leu
115 120
Leu Pro Ala Val Ser Ser Gly Lys Asn Ile
125 130
Lys Arg Thr Leu Ala Ala Met Pro Glu Glu
135 140
Glu Thr Thr Glu Ala Asn Ala Gly Gln Phe
145 150
Leu Ser Phe Ala Ser Leu Phe Leu Pro Lys
155 160
Leu Val Val Gly Glu Lys Ala Cys Leu Glu
165 170
Lys Val Gln Arg Gln Ile Gln Val His Ala
175 180
Glu Gln Gly Leu Ile Gln Tyr Pro Thr Ala
185 190
Trp Gln Ser Val Gly His Met Met Val Ile
195 200
Phe Arg Leu Met Arg Thr Asn Phe Leu Ile
205 210
Lys Phe Leu Leu Ile His Gln Gly Met His
215 220
Met Val Ala Gly His Asp Ala Asn Asp Ala
225 230
Val Ile Ser Asn Ser Val Ala Gln Ala Arg
235 240
Phe Ser Gly Leu Leu Ile Val Lys Thr Val
245 250
Leu Asp His Ile Leu Gln Lys Thr Glu Arg
255 260
Gly Val Arg Leu His Pro Leu Ala Arg Thr
265 270
Ala Lys Val Lys Asn Glu Val Asn Ser Phe
275 280
Lys Ala Ala Leu Ser Ser Leu Ala Lys His
285 290
Gly Glu Tyr Ala Pro Phe Ala Arg Leu Leu

295 300
Asn Leu Ser Gly Val Asn Asn Leu Glu His
305 310
Gly Leu Phe Pro Gln Leu Ser Ala Ile Ala
315 320
Leu Gly Val Ala Thr Ala His Gly Ser Thr
325 330
Leu Ala Gly Val Asn Val Gly Glu Gln Tyr
335 340
Gln Gln Leu Arg Glu Ala Ala Thr Glu Ala
345 350
Glu Lys Gln Leu Gln Gln Tyr Ala Glu Ser
355 360
Arg Glu Leu Asp His Leu Gly Leu Asp Asp
365 370
Gln Glu Lys Lys Ile Leu Met Asn Phe His
375 380
Gln Lys Lys Asn Glu Ile Ser Phe Gln Gln
385 390
Thr Asn Ala Met Val Thr Leu Arg Lys Glu
395 400
Arg Leu Ala Lys Leu Thr Glu Ala Ile Thr
405 410
Ala Ala Ser Leu Pro Lys Thr Ser Gly His
415 420
Tyr Asp Asp Asp Asp Asp Ile Pro Phe Pro
425 430
Gly Pro Ile Asn Asp Asp Asp Asn Pro Gly
435 440
His Gln Asp Asp Asp Pro Thr Asp Ser Gln
445 450
Asp Thr Thr Ile Pro Asp Val Val Val Asp
455 460
Pro Asp Asp Gly Ser Tyr Gly Glu Tyr Gln
465 470
Ser Tyr Ser Glu Asn Gly Met Asn Ala Pro
475 480
Asp Asp Leu Val Leu Phe Asp Leu Asp Glu
485 490
Asp Asp Glu Asp Thr Lys Pro Val Pro Asn
495 500
Arg Ser Thr Lys Gly Gly Gln Gln Lys Asn
505 510
Ser Gln Lys Gly Gln His Ile Glu Gly Arg
515 520
Gln Thr Gln Ser Arg Pro Ile Gln Asn Val
525 530
Pro Gly Pro His Arg Thr Ile His His Ala
535 540
Ser Ala Pro Leu Thr Asp Asn Asp Arg Arg
545 550
Asn Glu Pro Ser Gly Ser Thr Ser Pro Arg
555 560
Met Leu Thr Pro Ile Asn Glu Glu Ala Asp

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                    565                          570
Pro Leu Asp Asp Ala Asp Asp Glu Thr Ser
                    575                          580
Ser Leu Pro Pro Leu Glu Ser Asp Asp Glu
                    585                          590
Glu Gln Asp Arg Asp Gly Thr Ser Asn Arg
                    595                          600
Thr Pro Thr Val Ala Pro Pro Ala Pro Val
                    605                          610
Tyr Arg Asp His Ser Glu Lys Lys Glu Leu
                    615                          620
Pro Gln Asp Glu Gln Gln Asp Gln Asp His
                    625                          630
Thr Gln Glu Ala Arg Asn Gln Asp Ser Asp
                    635                          640
Asn Thr Gln Ser Glu His Ser Phe Glu Glu
                    645                          650
Met Tyr Arg His Ile Leu Arg Ser Gln Gly
                    655                          660
Pro Phe Asp Ala Val Leu Tyr Tyr His Met
                    665                          670
Met Lys Asp Glu Pro Val Val Phe Ser Thr
                    675                          680
Ser Asp Gly Lys Glu Tyr Thr Tyr Pro Asp
                    685                          690
Ser Leu Glu Glu Glu Tyr Pro Pro Trp Leu
                    695                          700
Thr Glu Lys Glu Ala Met Asn Glu Glu Asn
                    705                          710
Arg Phe Val Thr Leu Asp Gly Gln Gln Phe
                    715                          720
Tyr Trp Pro Val Met Asn His Lys Asn Lys
                    725                          730
Phe Met Ala Ile Leu Gln His His Gln
                    735
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<211> 251

<212> PRT

<213> Ebola Zaire

<220>

<400> 19

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Met Ala Lys Ala Thr Gly Arg Tyr Asn Leu
 1                  5                   10
Ile Ser Pro Lys Lys Asp Leu Glu Lys Gly
                    15                  20
Val Val Leu Ser Asp Leu Cys Asn Phe Leu
                    25                  30
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Val Ser Gln Thr Ile Gln Gly Trp Lys Val
35 40
Tyr Trp Ala Gly Ile Glu Phe Asp Val Thr
45 50
His Lys Gly Met Ala Leu Leu His Arg Leu
55 60
Lys Thr Asn Asp Phe Ala Pro Ala Trp Ser
65 70
Met Thr Arg Asn Leu Phe Pro His Leu Phe
75 80
Gln Asn Pro Asn Ser Thr Ile Glu Ser Pro
85 90
Leu Trp Ala Leu Arg Val Ile Leu Ala Ala
95 100
Gly Ile Gln Asp Gln Leu Ile Asp Gln Ser
105 110
Leu Ile Glu Pro Leu Ala Gly Ala Leu Gly
115 120
Leu Ile Ser Asp Trp Leu Leu Thr Thr Asn
125 130
Thr Asn His Phe Asn Met Arg Thr Gln Arg
135 140
Val Lys Glu Gln Leu Ser Leu Lys Met Leu
145 150
Ser Leu Ile Arg Ser Asn Ile Leu Lys Phe
155 160
Ile Asn Lys Leu Asp Ala Leu His Val Val
165 170
Asn Tyr Asn Gly Leu Leu Ser Ser Ile Glu
175 180
Ile Gly Thr Gln Asn His Thr Ile Ile Ile
185 190
Thr Arg Thr Asn Met Gly Phe Leu Val Glu
195 200
Leu Gln Glu Pro Asp Lys Ser Ala Met Asn
205 210
Arg Met Lys Pro Gly Pro Ala Lys Phe Ser
215 220
Leu Leu His Glu Ser Thr Leu Lys Ala Phe
225 230
Thr Gln Gly Ser Ser Thr Arg Met Gln Ser
235 240
Leu Ile Leu Glu Phe Asn Ser Ser Leu Ala
245 250
Ile

<210> 20

<211> 324

<212> PRT

<213> Ebola Zaire

<220>

<400> 20

Met Glu Ala Ser Tyr Glu Arg Gly Arg Pro
1 5 10
Arg Ala Ala Arg Gln His Ser Arg Asp Gly
15 20
His Asp His His Val Arg Ala Arg Ser Ser
25 30
Ser Arg Glu Asn Tyr Arg Gly Glu Tyr Arg
35 40
Gln Ser Arg Ser Ala Ser Gln Val Arg Val
45 50
Pro Thr Val Phe His Lys Lys Arg Val Glu
55 60
Pro Leu Thr Val Pro Pro Ala Pro Lys Asp
65 70
Ile Cys Pro Thr Leu Lys Lys Gly Phe Leu
75 80
Cys Asp Ser Ser Phe Cys Lys Lys Asp His
85 90
Gln Leu Glu Ser Leu Thr Asp Arg Glu Leu
95 100
Leu Leu Leu Ile Ala Arg Lys Thr Cys Gly
105 110
Ser Val Glu Gln Gln Leu Asn Ile Thr Ala
115 120
Pro Lys Asp Ser Arg Leu Ala Asn Pro Thr
125 130
Ala Asp Asp Phe Gln Gln Glu Glu Gly Pro
135 140
Lys Ile Thr Leu Leu Thr Leu Ile Lys Thr
145 150
Ala Glu His Trp Ala Arg Gln Asp Ile Arg
155 160
Thr Ile Glu Asp Ser Lys Leu Arg Ala Leu
165 170
Leu Thr Leu Cys Ala Val Met Thr Arg Lys
175 180
Phe Ser Lys Ser Gln Leu Ser Leu Leu Cys
185 190
Glu Thr His Leu Arg Arg Glu Gly Leu Gly
195 200
Gln Asp Gln Ala Glu Pro Val Leu Glu Val
205 210
Tyr Gln Arg Leu His Ser Asp Lys Gly Gly
215 220
Ser Phe Glu Ala Ala Leu Trp Gln Gln Trp
225 230
Asp Leu Gln Ser Leu Ile Met Phe Ile Thr
235 240

Pro Ile Asp Pro Thr Lys Asn Ile Met Gly
255 260
Ile Glu Val Pro Glu Thr Leu Val His Lys
265 270
Leu Thr Gly Lys Lys Val Thr Ser Lys Asn
275 280
Gly Gln Pro Ile Ile Pro Val Leu Leu Pro
285 290
Lys Tyr Ile Gly Leu Asp Pro Val Ala Pro
295 300
Gly Asp Leu Thr Met Val Ile Thr Gln Asp
305 310
Cys Asp Thr Cys His Ser Pro Ala Ser Leu
315 320
Pro Ala Val Ile Glu Lys
325

<210> 23

<211> 288

<212> PRT

<213> Ebola Zaire

<220>

<400> 23

Met Glu Ala Ser Tyr Glu Arg Gly Arg Pro
1 5 10
Arg Ala Ala Arg Gln His Ser Arg Asp Gly
15 20
His Asp His His Val Arg Ala Arg Ser Ser
25 30
Ser Arg Glu Asn Tyr Arg Gly Glu Tyr Arg
35 40
Gln Ser Arg Ser Ala Ser Gln Val Arg Val
45 50
Pro Thr Val Phe His Lys Lys Arg Val Glu
55 60
Pro Leu Thr Val Pro Pro Ala Pro Lys Asp
65 70
Ile Cys Pro Thr Leu Lys Lys Gly Phe Leu
75 80
Cys Asp Ser Ser Phe Cys Lys Lys Asp His
85 90
Gln Leu Glu Ser Leu Thr Asp Arg Glu Leu
95 100
Leu Leu Leu Ile Ala Arg Lys Thr Cys Gly
105 110
Ser Val Glu Gln Gln Leu Asn Ile Thr Ala
115 120
Pro Lys Asp Ser Arg Leu Ala Asn Pro Thr
125 130

```
Ala Asp Asp Phe Gln Gln Glu Glu Gly Pro
                135                 140
Lys Ile Thr Leu Leu Thr Leu Ile Lys Thr
                145                 150
Ala Glu His Trp Ala Arg Gln Asp Ile Arg
                155                 160
Thr Ile Glu Asp Ser Lys Leu Arg Ala Leu
                165                 170
Leu Thr Leu Cys Ala Val Met Thr Arg Lys
                175                 180
Phe Ser Lys Ser Gln Leu Ser Leu Leu Cys
                185                 190
Glu Thr His Leu Arg Arg Glu Gly Leu Gly
                195                 200
Gln Asp Gln Ala Glu Pro Val Leu Glu Val
                205                 210
Tyr Gln Arg Leu His Ser Asp Lys Gly Gly
                215                 220
Ser Phe Glu Ala Ala Leu Trp Gln Gln Trp
                225                 230
Asp Arg Gln Ser Leu Ile Met Phe Ile Thr
                235                 240
Ala Phe Leu Asn Ile Ala Leu Gln Leu Pro
                245                 250
Cys Glu Ser Ser Ala Val Val Val Ser Gly
                255                 260
Leu Arg Thr Leu Val Pro Gln Ser Asp Asn
                265                 270
Glu Glu Ala Ser Thr Asn Pro Gly Thr Cys
                275                 280
Ser Trp Ser Asp Glu Gly Thr Pro
                285
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<210> 24

<211> 11

<212> PRT

<213> Ebola Zaire

<220>

<400> 24

```
Val Tyr Gln Val Asn Asn Leu Glu Glu Ile
 1              5                   10
Cys
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<210> 25

<211> 23

<212> PRT

<213> Ebola Zaire

<220>

<400> 25

```
Leu Lys Phe Ile Asn Lys Leu Asp Ala Leu
 1               5                   10
Leu Val Val Asn Tyr Asn Gly Leu Leu Ser
                15                   20
Ser Ile Phe
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